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Development and Enhancement of Liquisolid Compact Containing Rifampicin and Quercetin: An In-Vitro and In-Vivo Investigation

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ABSTRACT

The main objective of this study was to develop liquisolid compacts containing rifampicin and quercetin with enhanced gastrointestinal absorption and dissolution characteristics. To achieve the desired formulations, non-volatile liquid carriers such as propylene glycol, PEG 200, and Tween 20 were selected due to their superior drug solubility properties. The liquisolid formulations were subsequently blended with carrier and coating materials to produce a free-flowing and compressible powder. Avicel pH-102, Aeroperl 200, and Aerosil 200 exhibited excellent liquid retention capacities, indicating their effectiveness as solid carriers and coating agents in liquisolid compact formulations. FT-IR spectra confirmed the absence of significant interactions between the drug and carrier. DSC and PXRD analyses showed that the crystalline structure of the drug was no longer present in the liquisolid powders. Furthermore, the enhanced dissolution behavior observed in liquisolid systems suggested that the drug transitioned into an amorphous or molecular state. Pharmacokinetic studies conducted in rats showed that the inclusion of non-volatile liquid carriers in liquisolid systems improves drug absorption, leading to an increase in both the gastrointestinal uptake and dissolution rate of rifampicin and quercetin.

Keywords: D-optimal design, Rifampicin (RIF), Liquisolid, Quercetin (QUE), Formulation

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Introduction

Dissolution, which is largely governed by a drug's solubility, plays a crucial role in determining its bioavailability. Since most newly developed pharmaceutical compounds exhibit low aqueous solubility or poor dissolution properties (BCS Class-II), dissolution becomes the rate-limiting step in their absorption process, leading to inadequate uptake. This challenge has posed significant difficulties for both the pharmaceutical industry and researchers striving to develop more effective drug formulations [1-6].

The physicochemical properties of drugs have a direct impact on their bioavailability and therapeutic efficacy. Therefore, enhancing the dissolution profile remains a critical research concern [1-6]. Various strategies have been explored in the literature to improve the oral bioavailability of rifampicin and quercetin, including solubilization using surfactant-based systems [7], solid dispersions [8-11], nanoparticles [12, 13], nanosuspensions [14], solid lipid particles [15-17], and inclusion complexes [18, 19].

However, each of these methods presents certain challenges during formulation. For example, polymeric nanoparticles require specialized equipment such as sonicators for preparation and spray dryers for drying, increasing production costs. Additionally, nanosuspensions, although initially exhibiting high solubility and rapid dissolution, tend to suffer from particle agglomeration, which diminishes their advantages.

Liquisolid technology has emerged as a promising alternative for achieving rapid and complete drug dissolution. This approach is not only straightforward but also cost-effective and scalable for improving the dissolution rates of poorly water-soluble drugs. In this technique, the drug is first dissolved in a non-volatile liquid vehicle and subsequently incorporated into carrier and coating materials, forming a free-flowing, non-adherent, and compressible powder. Once the liquid drug saturates the carrier matrix, a thin coating layer forms, absorbing any residual liquid and ensuring that the final system remains dry and flowable. The liquisolid method has shown significant potential in enhancing the dissolution profiles of numerous drugs [3, 20-24].

By increasing the net effective surface area and facilitating the wetting process, liquisolid compacts significantly enhance the dissolution rate of poorly soluble drugs, thereby improving their availability in dissolution media [3, 20-24].

Rifampicin, a semi-synthetic antibiotic derived from *Streptomyces mediterranei*, exhibits a broad-spectrum antibacterial effect, particularly against *Mycobacterium* species. Its mechanism of action involves the inhibition of DNA-dependent RNA polymerase by forming a stable complex with the enzyme, thereby preventing the initiation of RNA synthesis in susceptible bacteria [25].

Despite its efficacy, oral rifampicin formulations have a bioavailability of only 50–60%, necessitating higher dosages to achieve therapeutic effects. Additionally, tuberculosis treatment regimens present several challenges, including prolonged treatment duration, the emergence of drug-resistant strains, poor patient adherence, immune suppression, and lung tissue damage [25]. High doses of rifampicin are also associated with adverse effects such as hepatotoxicity, neurotoxicity, renal impairment, hematological disorders, and other systemic complications [25].

Quercetin has been reported in the literature as a bioenhancer capable of increasing drug bioavailability while reducing the side effects associated with conventional tuberculosis therapy. Herbal bio-enhancers have been widely used to improve the bioavailability and therapeutic efficacy of various drug classes, including antibiotics, antifungals, antivirals, and anti-tubercular agents [26, 27]. The inclusion of bio-enhancers is intended to reduce the required drug dosage, minimize toxicity, and shorten treatment duration. Furthermore, quercetin's hepatoprotective and immunomodulatory properties provide additional therapeutic benefits in tuberculosis treatment [9, 10].

A naturally occurring polyphenolic flavonoid found in citrus fruits, vegetables, and plants, quercetin functions by inhibiting the CYP3A4 enzyme and the P-gp efflux pump. It also possesses multiple pharmacological activities, including antioxidant, anti-inflammatory, anti-allergic, antibacterial, anti-tubercular, anti-neoplastic, and anti-atherosclerotic effects. Studies have demonstrated that quercetin enhances the bioavailability, plasma concentration, and therapeutic efficacy of several drugs, such as diltiazem, digoxin, verapamil, etoposide, and paclitaxel [28, 29].

Based on these considerations, this research aimed to improve the dissolution of rifampicin by utilizing quercetin as a bioenhancer, employing the liquisolid compact technology in conjunction with the design of experiments (DoE) approach. Using Design-Expert software, the solubility characteristics and percentage cumulative drug release of liquisolid compacts were systematically analyzed, leading to the selection of an optimal formulation based on the desirability function.

To determine the appropriate quercetin dosage, an in-vitro anti-tuberculosis bioassay was conducted using different rifampicin-to-quercetin ratios (1:1 and 1:0.5). The findings indicated that the 1:1 ratio exhibited significant activity against *Mycobacterium tuberculosis* H37Rv strain. Consequently, 150 mg of rifampicin and 150 mg of quercetin were selected for further formulation development [30].

Materials and Methods

Materials

Analytical-grade rifampicin and quercetin were procured from Swapnroop Drugs and Pharmaceuticals, Aurangabad, Maharashtra, India. Aeroperl 200 and Aerosil 200 were received as complimentary samples from Evonik Pharma, Mumbai, India. Additionally, Neusilin, Syloid XDP 3150, and Syloid 244 FP were obtained from Company KG, Germany. Hydrochloric acid, sodium hydroxide, and potassium hydrogen orthophosphate were supplied by Merck, Mumbai, India. A 0.1 M hydrochloric acid solution and a pH 6.8 phosphate buffer were prepared following the Indian Pharmacopeia guidelines. Distilled water was utilized throughout the study.

Solubility assessment

To assess saturation solubility, various non-volatile solvents—including propylene glycol, Tween 20, Tween 80, and polyethylene glycol (PEG 200, 400, and 600)—were evaluated. Excess amounts of rifampicin and quercetin were introduced into 1 ml of the chosen solvent and vortex-mixed for 15 minutes using a Remi vortex mixer (India). The mixtures were then centrifuged at 5000 rpm for fifteen minutes. After separation, the supernatant was collected and filtered through a 0.45 µm membrane filter. The clear filtrates were diluted with methanol, and drug concentrations were quantified using a UV spectrophotometer (Shimadzu Corp., Japan) at 365 nm and 470 nm for quercetin and rifampicin, respectively. Each experiment was conducted in triplicate [3, 20-24].

Optimization of non-volatile vehicle composition using d-optimal mixture design

Solubility data indicated that propylene glycol, Tween 20, and PEG 200 provided the highest drug solubilization. To maximize drug dissolution, various combinations of these solvents were tested. Based on initial trials, the optimal solvent composition required further refinement within specific concentration ranges: Tween 20 (0.1–0.2 ml), PEG 200 (0.3–0.7 ml), and propylene glycol (0.2–0.6 ml). The solubilized drug content depends on the solvent's capacity to retain the drug in solution, making the optimization of this mixture crucial. Therefore, a D-optimal mixture design was employed for refinement. Experimental data were analyzed using Design Expert version 11, generating 16 randomized trials. Independent variables included Tween 20, PEG 200, and propylene glycol (X1, X2, and X3), while the response variables were drug solubility (mg/ml) and percentage cumulative drug release (CDR) at 60 minutes in 0.1 M hydrochloric acid. These factors were considered critical in enhancing the oral bioavailability of poorly water-soluble drugs in liquisolid compact formulations [3, 20-24].

Measurement of flowable liquid-retention potential (Φ-value)

A powder sample (5 g) was gradually mixed with the liquid drug, and the resulting blend was placed at one end of a polished metal plate. The plate was incrementally inclined while keeping one edge on the ground, and the angle at which the blend began to slide was recorded. A slide angle of approximately 33° was considered indicative of optimal flow properties, ensuring the powder excipient remained well-suited for the chosen liquid vehicle [3, 20-24].

Determination of liquid load factor

Once the Φ-value of the carrier and coating materials was established, the corresponding liquid load factor required for achieving acceptable flow behavior was computed using the relevant equations.

$$Lf = (CA + CO)/R \quad (1)$$

$$R = Q/q \quad (2)$$

The ability of carrier and coating materials to retain liquid while maintaining flowability was assessed, represented as CA for carriers and CO for coating agents. The excipient ratio (R), determined using Eq. 2, plays a crucial role in optimizing flow properties. Previous research has demonstrated that an R-value within the range of 10 to 20 yields superior flow characteristics. Based on these findings, an average value of 15 was selected for this study's calculations [3, 20-24].

Evaluation of carrier and coating materials for non-volatile solvents

A preliminary assessment was conducted using Avicel pH-102, Lactose, Sorbitol, Dicalcium Phosphate, Aeroperl 200, Neusilin, Syloid XDP 3150, and Syloid 244 FP as potential carrier materials, while Aerosil 200 was examined as the coating agent. The selection process was guided by literature reviews. Trials revealed that Avicel pH-102, Lactose, Sorbitol, and Dicalcium Phosphate required more than 1 g to fully adsorb one ml of liquid medication, making them unsuitable for oral formulations. Additionally, the quantities necessary for Aeroperl 200, Neusilin, Syloid XDP 3150, and Syloid 244 FP surpassed IIG limitations. Consequently, different combinations of carrier and coating materials were explored. The angle of repose (θ) was measured to determine the flow properties of the resulting liquisolid systems [3, 20-24].

Formulation of liquisolid systems

The formulation process began by dissolving the drug in the optimized non-volatile solvent mixture inside five ml RIA vials, ensuring complete solubilization through vortex mixing. The liquisolid formulation was then prepared following the methodology established by Spireas *et al*. which involves three distinct stages. First, the liquid medication was gradually incorporated into the selected carrier and coating materials and blended at a rate of approximately one rotation per second for one minute to ensure uniform dispersion. In the second phase, the mixture was spread evenly across the mortar's surface and left undisturbed for five minutes, allowing the drug solution to integrate into the excipient matrix. The final stage involved carefully scraping the powder from the mortar using an aluminum spatula, followed by an additional 30-second mixing with a lubricant and glidant. The completed liquisolid formulation was encapsulated in size 00 gelatin capsules [3, 20-24].

Dissolution testing in vitro

Dissolution profiles of the developed liquisolid formulations were evaluated against commercial RIF (R-cin 150, one hundred fifty mg rifampicin capsule IP, Lupin Pharmaceutical Pvt, Ltd.) and QUE (quercetin 100 mg capsule, HealthVita) using a USP Type I dissolution apparatus. The dissolution study was conducted in 900 ml of either 0.1 M hydrochloric acid or a 0.1 M phosphate buffer at pH 6.8, supplemented with 0.02% vitamin C and 0.2% SLS. The apparatus was set to 50 rpm at a temperature of 37 ± 0.5 °C. At predefined time points (0, 15, 30, 45, and 60 minutes), samples were withdrawn, filtered using Whatman filter paper, and appropriately diluted with the dissolution medium. All experiments were conducted under sink conditions. The collected samples were analyzed using a newly developed UV-visible spectrophotometric technique. Additionally, dissolution performance was evaluated by calculating the dissimilarity factor (f1) and similarity factor (f2) using standard equations [3, 20-24].

$$f_1 = \frac{\sum R_t - T_t}{\sum R_t} \times 100 \quad (3)$$

$$f_2 = 50 \times \left\{ log \left[1 + \left(\frac{1}{n}\right) \sum_{t=1}^{n} |R_t - T_t|^2 \right] - 0.5 \times 100 \right\} \quad (4)$$

Where Rt is drug release from reference at time t, Tt is drug release from reference at time t, n = number of sampling points.

Drug-excipient compatibility evaluation

FT-IR spectroscopy

To assess potential interactions between the drug and excipients, FT-IR spectra of rifampicin, quercetin, Avicel pH-102, Aeroperl 200, and the optimized liquisolid formulation were recorded using a spectrophotometer (Bruker Optics, USA). The KBr pellet method was applied, scanning within the 4000–500 cm^{-1} range at a resolution of 4 cm^{-1} [3, 20-24].

DSC analysis

The thermal properties of rifampicin, quercetin, Avicel pH-102, Aeroperl 200, and the optimized liquisolid formulation were examined using a differential scanning calorimeter (Perkin Elmer, USA). Samples were sealed in aluminum crucibles and subjected to a temperature range of 20 to 400 °C at a heating rate of 10 °C per minute under a controlled nitrogen flow of 30 ml/min [3, 20-24].

PXRD analysis

To determine the crystalline nature of the drug within the formulation, PXRD studies were performed. The diffraction patterns of the pure drug, excipients, and liquisolid formulation were obtained with an X-ray diffractometer (Bruker D2 Phaser) operating at 30 kV and 10 mA using CuK radiation and an X'celerator detector [3, 20-24].

Stability assessment

The optimized liquisolid formulation was stored at 40°C with 75% relative humidity for three months. Its physical properties, including hardness and disintegration time, were assessed alongside its drug release profile at 15, 30,

45, and 60 minutes. The dissolution characteristics of the aged formulation were compared with those of a freshly prepared sample [3, 20-24].

In-vivo pharmacokinetic study in rats

The pharmacokinetic study was conducted following approval from Anand Pharmacy College's Institutional Animal Ethics Committee (IAEC) (Registration No. 277/PO/ReBi/2000/CPCSEA) and the Committee for Control and Supervision of Experiments on Animals (CPCSEA) (Protocol No. 1928, dated 11 October 2019). Wistar albino rats (weighing approximately 250 ± 10 g) were housed under controlled conditions (20 ± 2°C) with a 12-hour light/dark cycle, using corn cob bedding. They underwent a 12-hour fasting period before receiving treatment, with free access to water.

Each rat was administered 4.0 mg/kg of RIF and QUE orally via gavage. The study included five groups (n = 4 per group), with further divisions into subgroups A and B. The control group received a CMC suspension, while the remaining groups were given pure RIF API, a combination of RIF and QUE APIs, a commercial RIF and QUE formulation, or the developed liquisolid formulation.

Blood samples were withdrawn from the retro-orbital vein at predefined intervals (0, 1, 2, 3, 4, 5, and 8 hours) and collected in Eppendorf tubes containing disodium EDTA. Plasma was separated through centrifugation at 5000 rpm for ten minutes and stored at -20 °C for later analysis. The concentrations of RIF and QUE in plasma were determined using UPLC-MS/MS within 6-7 hours of sample collection. Pharmacokinetic parameters were calculated through a non-compartmental approach, and the AUC (area under the plasma concentration-time curve) was determined using the linear trapezoidal method for pure APIs, marketed formulations, and the optimized liquisolid system [3, 20-24].

Results and Discussion

Solubility evaluation

To select an appropriate liquid carrier for RIF and QUE liquisolid compacts, solubility assessments were conducted in multiple non-volatile solvents. Identifying the maximum solubility of the drug in these vehicles helped in optimizing the formulation by minimizing the risk of drug precipitation in vivo and reducing the overall weight of the final product. Poorly soluble drugs tend to dissolve better in medium-chain triglycerides and mono- or diglycerides due to their polarity. Among the tested non-ionic surfactants, propylene glycol (25 milligrams per milliliter for RIF and 35 milligrams per milliliter for QUE), PEG 200 (25 milligrams per milliliter for RIF and 40 milligrams per milliliter for QUE), and Tween 20 (ten milligrams per milliliter for RIF and eight mg/ml for QUE) exhibited superior solubility potential.

In contrast, the solubility of RIF and QUE in Tween 80 (5 milligrams per milliliter for RIF and 7 milligrams per milliliter for QUE), PEG 400 (fifteen milligrams per milliliter for RIF and twenty milligrams per milliliter for QUE), and PEG 600 (ten mg/ml for both) was inadequate for direct dissolution. Consequently, PEG 200 was selected to dissolve the drug, followed by the addition of Tween 20 as a surfactant, with propylene glycol functioning as the solvent.

Optimization of non-volatile vehicle composition

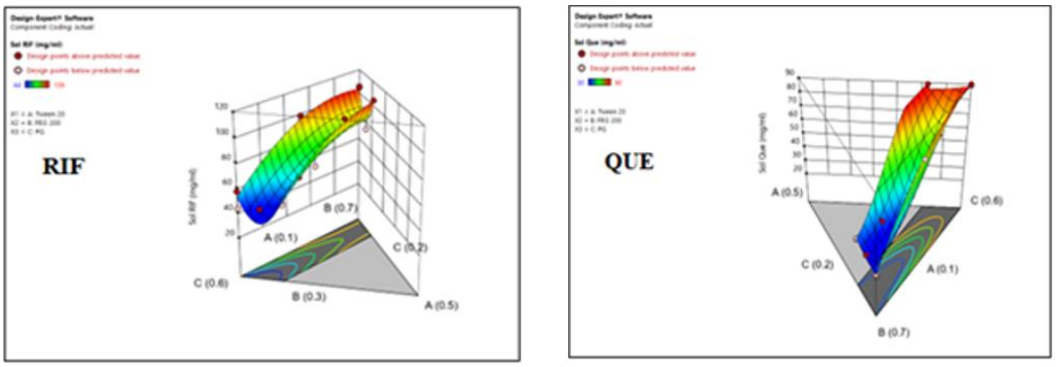
The experimental design based on the D-optimal mixture approach is outlined in **Table 1**, detailing the influence of variable X on the observed Y responses.

Table 1. The influence of D-optimal design output (16 batches) independent variables (X1 to X3) and independence variables (Y1 and Y2)

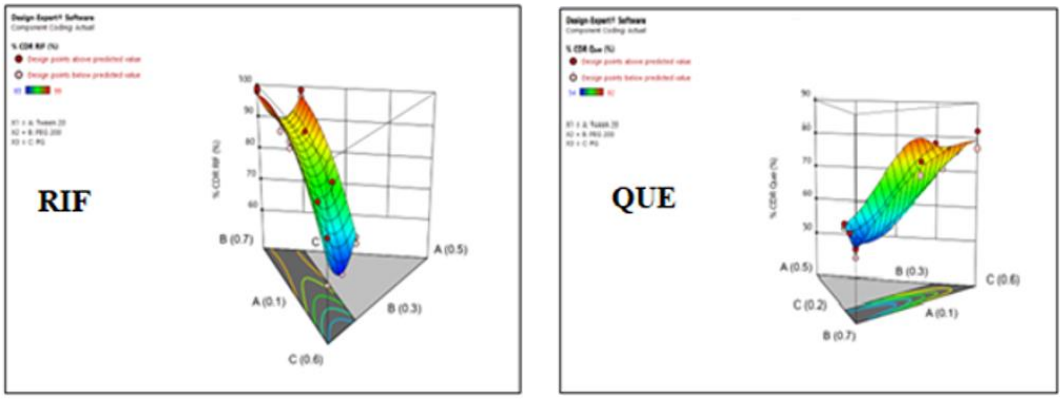
Batch	Tween 20 (ml) X1	PEG 200 (ml) X2	PG. (ml) X3	Solubility (mg/ml) Y1		% Drug release at 60 min Y2	
				RIF	QUE	RIF	QUE
1	0.20	0.30	0.50	52	85	73	76
2	0.17	0.39	0.44	62	75	85	74
3	0.12	0.39	0.49	60	70	82	72
4	0.20	0.30	0.50	50	89	71	78
5	0.20	0.60	0.20	105	35	99	60

6	0.10	0.30	0.60	58	90	79	82
7	0.15	0.47	0.37	61	41	82	63
8	0.10	0.70	0.20	106	31	99	54
9	0.10	0.70	0.20	105	30	98	56
10	0.20	0.60	0.20	105	34	97	59
11	0.15	0.30	0.55	45	84	65	76
12	0.10	0.30	0.60	44	89	65	77
13	0.10	0.50	0.40	99	74	94	72
14	0.17	0.54	0.28	95	35	91	60
15	0.10	0.50	0.40	96	79	92	76
16	0.15	0.65	0.20	75	34	86	59

The three-dimensional contour plot **(Figure 1a)** illustrates that elevating PEG 200 and propylene glycol concentrations enhances the solubility of RIF and QUE. Likewise, **Figure 1b** reveals that the percentage of cumulative drug release for both compounds rises as the levels of PEG 200 and propylene glycol increase [3, 20-24].



a)



b)

Figure 1. a) 3D contour plot of % CDR of RIF and QUE at 60 min, and b) 3D contour plot of Solubility of RIF and QUE

A quadratic model was identified as the best fit for RIF, while a special quartic model provided the closest match for QUE, with adjusted R^2 values of 0.9323 and 0.9958, respectively. Model validation was carried out using analysis of variance (ANOVA), confirming that all statistical parameters fell within the optimal range. The coefficient of variation (% CV) was 5.12 for RIF and 3.09 for QUE, both < 10%, while adequate precision values were 14.88 for RIF and 32.76 for QUE, exceeding the threshold of 10. The P-value of 0.0001 for both RIF and QUE was under 0.05, indicating statistical significance for solubility and cumulative drug release (% CDR).

To optimize desirability, constraints were applied to enhance solubility and % CDR based on statistical evaluation and ANOVA findings. The Design-Expert program suggested three experimental runs for validation, with Batch-B [Tween 20 (0.1 milliliter): PEG 200 (0.462 milliliter): PG (0.437 milliliter)] being selected due to its minimal error compared to the other two batches.

Flowable liquid-retention potential

Among the tested carriers, Aeroperl demonstrated the highest capacity for retaining flowable liquid at 1.98 milliliters per gram while maintaining good flow properties (angle of slide = 34). However, regulatory guidelines limit the maximum daily intake of Aeroperl to 200 milligrams, while over 500 milligrams would be required for liquid adsorption in the formulation. Consequently, Aeroperl, Syloid 244 EP, Syloid XDP 3150, and Neusilin US2 could not be used independently for liquid absorption. A secondary carrier with a retention potential of 0.76 milliliters was introduced, leading to further investigations into different carrier ratios to ensure effective adsorption and optimal flowability. Among coating materials, Aerosil exhibited the highest retention capacity at 1.922 milliliters.

Liquid load factor

The liquid load factor was determined using Eq. 1. For Avicel pH-102/Aeroperl 200 in a 5:1 ratio, the corresponding -value was calculated as 1.15 milliliters. Meanwhile, the selected coating material, Aerosil 200, achieved a value of 1.92. With R determined as 6, substitution into Eq. 1 yielded a liquid load factor (Lf) of 0.51, indicating the feasibility of incorporating a significant amount of liquid medication into the powder blend.

Selection of carrier and coating materials for the non-volatile vehicle

Based on **Table 2**, Avicel pH-102 and Aeroperl 200 were identified as the most suitable carriers, while Aerosil 200 was chosen as the optimal coating material to accommodate 1 milliliter of liquid. To improve powder flow properties, magnesium stearate and talc were incorporated into the formulation. The final powder blend was encapsulated into 00-size capsules for commercial viability [3, 20-24].

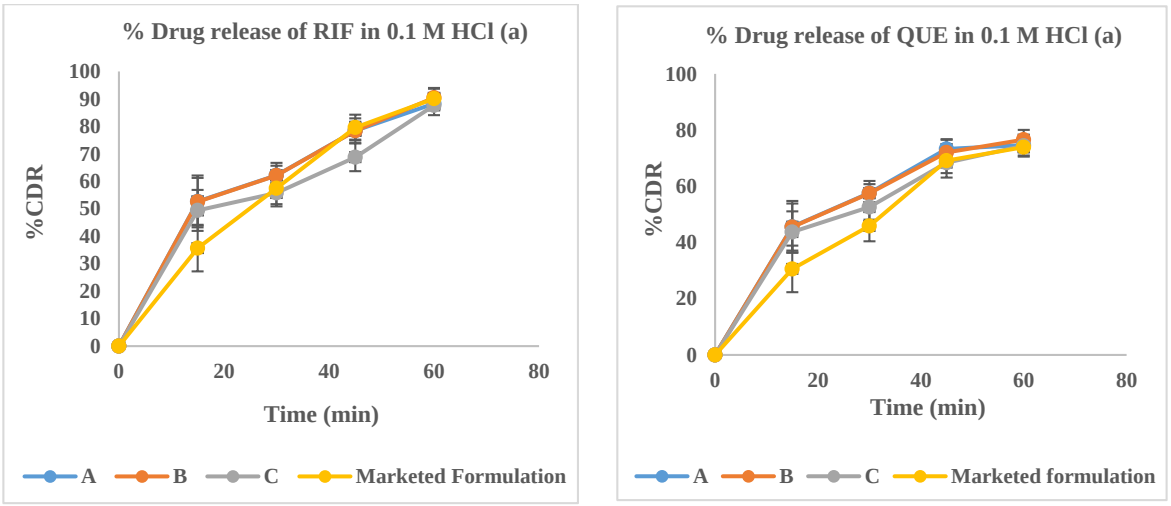
Table 2. Selection of carrier and coating material based on preliminary batches

Batch	Avicel pH 102 (mg)	Lactose (mg)	DCP (mg)	Aeroperl 200 (mg)	Neusilin (mg)	Syloid XDP 3150 (mg)	Syloid 244 FP (mg)	Aerosil 200 (mg)	Magnesium stearate and talc (1:1) (mg)	Total weight (mg)	Flow property (angle of repose) θ
1	500	-	-	100	-	-	-	100	150	850	27
2	550	-	-	-	150	-	-	100	150	950	31
3	580	-	-	-	-	100	-	100	150	930	30
4	590	-	-	-	-	-	100	100	150	940	28
5	-	600	-	100	-	-	-	100	150	950	28
6	-	620	-	-	150	-	-	100	150	1020	28
7	-	610	-	-	-	100	-	100	150	960	29
8	-	610	-	-	-	-	100	100	150	960	29
9	-	-	630	100	-	-	-	100	150	980	27
10	-	-	640	-	150	-	-	100	150	1040	26
11	-	-	635	-	-	100	-	100	150	985	27
12	-	-	635	-	-	-	100	100	150	985	28

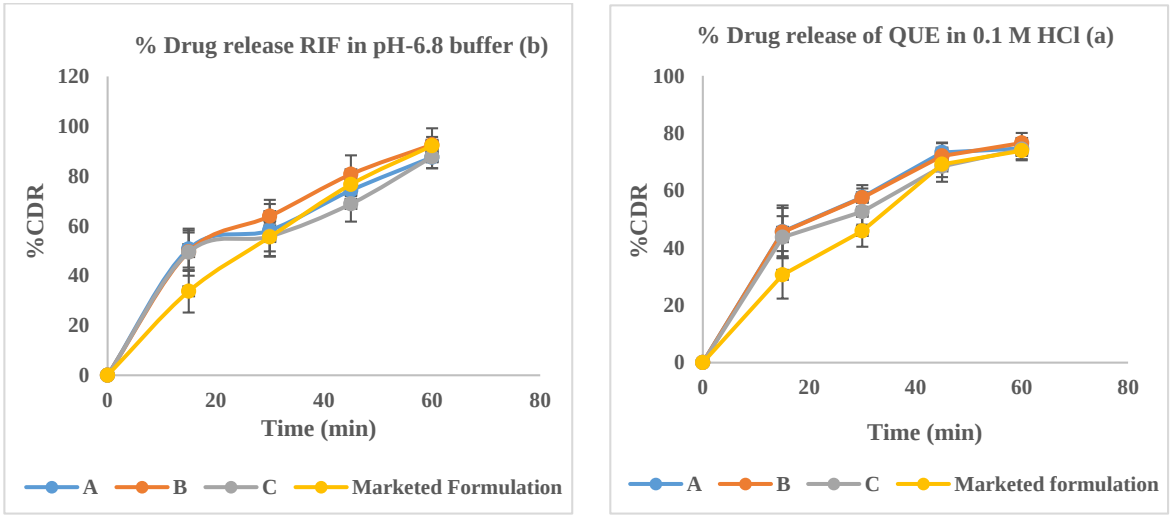
In-vitro dissolution studies

The release profile of RIF and QUE was evaluated in both 0.1 molar hydrochloric acid and phosphate buffer at pH 6.8. For RIF, drug release exceeded 85% within 60 minutes in 0.1 molar hydrochloric acid and surpassed 90%

in the phosphate buffer. Similarly, QUE exhibited a drug release of more than 75% after 60 minutes in both dissolution media. The commercial formulations of both drugs demonstrated a comparable release pattern, as illustrated in **Figures 2a and 2b**.

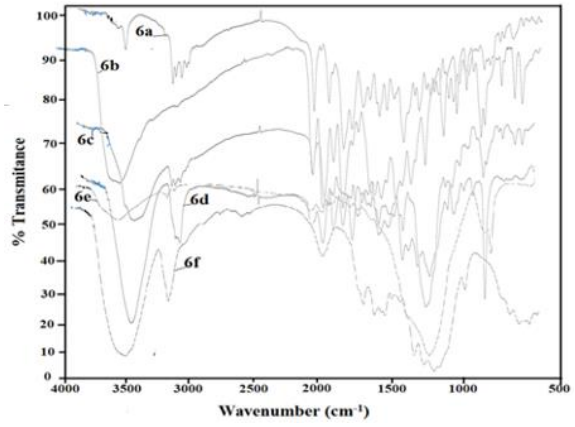


a)

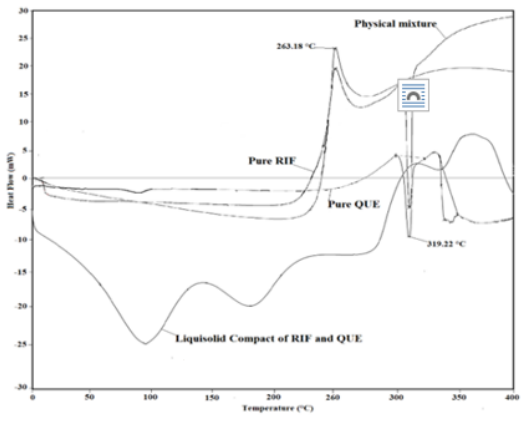


b)

Figure 2. a) the dissolution profile of RIF and QUE marketed product (MP) and test product (TP) in pH-6.8 phosphate buffer, and b) the dissolution profile of RIF and QUE marketed product (MP) and test product (TP) in 0.1 M HCl



a)



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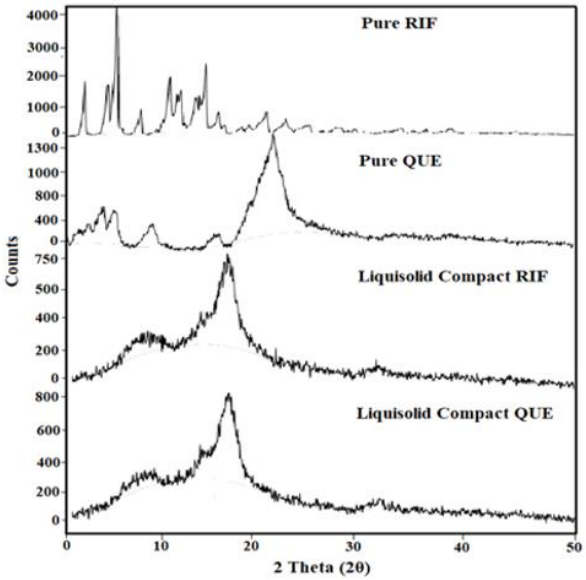


c)

Figure 3. a) FT-IR spectra of pure rifampicin, quercetin, and liqui-solid formulation, b) DSC thermogram of pure rifampicin, a) quercetin, b) liqui-solid formulation, c) Avicel pH-102, d) Aeroperl 200, e) and c) PXRD spectra of pure rifampicin, quercetin, Aeroperl 200 and Liqui-solid formulation and Avicel pH-102.

Compatibility assessment of drug and excipients

Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectra illustrated in **Figure 3a** compare RIF, QUE, Avicel PH 102, Aeroperl 200, their physical mixture, and the final liquisolid compact. No major spectral variations were observed between the liquisolid formulations and the pure drugs, indicating that incorporating rifampicin and quercetin into the non-volatile liquid did not alter the positioning of their functional groups. The absence of notable shifts in FT-IR peaks, along with the retention of the characteristic drug peaks in the liquisolid formulation, confirms that there was no significant interaction between the drugs and the excipients.

Differential scanning calorimetry (DSC) analysis

The DSC thermograms, presented in **Figure 3b**, reveal the thermal behavior of RIF, QUE, Avicel PH 102, Aeroperl 200, their physical mixture, and the liquisolid compact. The thermograms confirm the crystalline structure of the drugs, with distinct endothermic peaks detected at 263.18 °C for rifampicin and 319.22 °C for quercetin, corresponding to their melting points. However, these distinctive peaks were no longer visible in the liquisolid compact, suggesting that the drugs had transitioned into a dissolved state. This disappearance indicates molecular dispersion of the active ingredients within the liquisolid system.

Powder X-ray diffraction (PXRD) analysis

PXRD analysis confirmed the crystalline structure of RIF, with pronounced diffraction peaks observed at angles (2θ) of 11.67°, 11.72°, 11.77°, 11.82°, and 11.87°. Similarly, QUE exhibited strong diffraction peaks at 11.21°, 11.77°, 11.87°, 12.38°, 12.53°, and 13.09°. Following adsorption onto Avicel PH 102 and Aeroperl 200 after dissolution in a non-volatile solvent, the liquisolid formulation still exhibited peaks at 11.72°, 11.77°, 11.87°, 12.38°, and 13.09°, but their intensity was considerably reduced. This decline suggests that the drugs were present in an amorphous or solubilized form. The diminished intensity of sharp diffraction peaks further supports this transformation, as depicted in **Figure 3c**.

Stability evaluation

The physicochemical properties of the liquisolid powder remained stable throughout the storage period, with no noticeable alterations, confirming its stability.

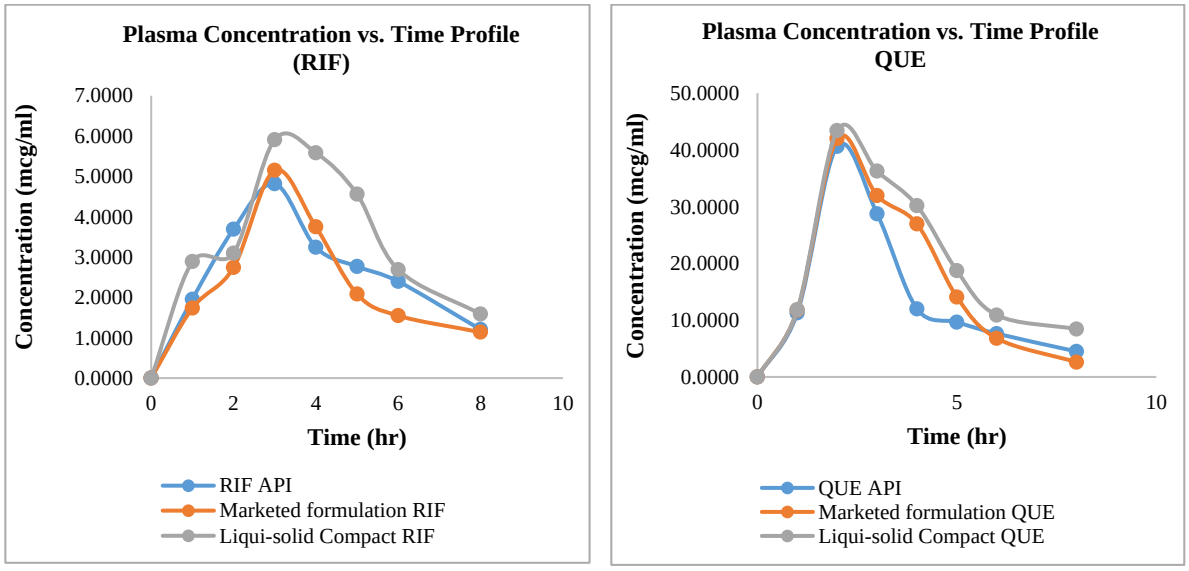
In-vivo pharmacokinetic investigation

The concentrations of RIF and QUE in rat plasma were determined using UPLC-MS/MS. Pharmacokinetic assessment revealed that drug absorption from the liquisolid compact was comparable to that of the marketed formulation. The maximum plasma concentration (Cmax) of RIF in the liquisolid compact was measured at 5.90 micrograms per milliliter, while the marketed formulation recorded a Cmax of 5.15 micrograms per milliliter. The findings indicated that the bioavailability and dissolution profile of the liquisolid formulation closely matched that of the commercial product, as shown in **Table 3** and **Figure 4**.

Table 3. Pharmacokinetic parameters of spherical agglomerates and marketed formulation

Pharmacokinetic parameters	Liqui-solid compact		Marketed formulation		Pure API	
	RIF	QUE	RIF	QUE	RIF	QUE
AUC_{0-t} (µg.hr/ml)	27.65	165.72	18.91	139.58	19.67	118.28
$AUMC_{0-t}$ (µg.hr/ml)	108.82	587.38	71.11	452.01	79.02	379.81
MRT (hr)	3.93	3.55	3.75	3.23	4.01	3.21
C_{max} (µg/ml)	5.90	43.42	5.15	41.98	4.32	40.63
t_{max} (hr)	3	2	3	2	3	2
K_{ab} (hr^{-1})	0.49	2.22	0.62	1.03	0.96	0.80
K_{el} (hr^{-1})	0.32	0.31	0.28	0.53	0.26	0.33
$t_{1/2}$ (Absorption) (hr)	1.40	0.31	1.11	0.67	0.72	0.86
$t_{1/2}$ (Elimination) (hr)	2.11	2.18	2.46	1.29	2.58	2.05

AUC_{0-t}, = area under the plasma concentration vs. time curve of last available measurement; $AUMC_{0-t}$, = area under the plasma concentration x time vs. time curve from 0 to time; MRT = mean residence time; C_{max} = maximum concentration; t_{max} = time of peak concentration; K_{ab} = absorption constant; K_{el} = elimination constant; $t_{1/2}$ = half-life



a) b)

Figure 4. Pharmacokinetic profile of RIF and QUE marketed product, pure API and liquisolid formulation in the rat.

Conclusion

The findings indicate that the liquisolid technique presents a promising approach for improving the dissolution characteristics of drugs with poor aqueous solubility and high dosage requirements. The incorporation of a non-volatile solvent and a surfactant into the liquisolid compact contributed to enhanced drug release. The application of a fractional factorial design demonstrated that varying combinations of PEG 200, Propylene glycol, and Tween 20 significantly influenced the dissolution behavior of the formulation. According to DSC and XRD analyses, the improved dissolution profile observed in the liquisolid compact can likely be attributed to the drug existing in an amorphous form or being molecularly dispersed within the formulation. Stability studies conducted over three

months confirmed that the essential properties of the formulation remained unchanged. Pharmacokinetic evaluation further demonstrated that the developed liquisolid formulation facilitated higher drug absorption into the bloodstream compared to the commercially available product.

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