RESEARCH PAPER

In-Vitro Comparative Evaluation and *In-Vivo* Pharmacokinetic Assessment of Tenofovir Disoproxil Fumarate Delivered via an Optimized SNEDDS Formulation

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ABSTRACT

Background: Tenofovir Disoproxil Fumarate (TDF), a first-line antiretroviral agent, suffers from poor aqueous solubility and limited oral bioavailability, compromising therapeutic efficacy. This study aimed to develop and evaluate a Self-Nanoemulsifying Drug Delivery System (SNEDDS) to enhance the dissolution and pharmacokinetic performance of TDF.

Methods: SNEDDS formulations were prepared using Capmul MCM as the oil phase, Tween 80 as the surfactant, and PEG 400 as the co-surfactant, followed by optimization through ternary phase diagram analysis. The optimized B4 formulation was characterized for droplet size, polydispersity index (PDI), zeta potential, and thermodynamic stability. In vivo pharmacokinetic evaluation was conducted in male Wistar rats, comparing the optimized SNEDDS with a pure drug suspension (20 mg/kg, oral). Plasma samples were analyzed by validated HPLC, and pharmacokinetic parameters were determined using non-compartmental analysis.

Results: The optimized SNEDDS exhibited a mean droplet size of 24.8 ± 1.2 nm, narrow PDI (0.212 ± 0.004), and negative zeta potential (−21.4 ± 0.7 mV), indicating high physical stability. In vivo, SNEDDS achieved a significantly higher Cmax (3.92 ± 0.28 µg/mL) compared to the pure drug (1.85 ± 0.21 µg/mL, $p < 0.01$) and a 2.3-fold increase in AUC0–∞, with reduced Tmax and extended half-life. These findings confirm enhanced oral absorption and sustained systemic exposure.

Conclusion: The developed TDF-SNEDDS markedly improved dissolution and bioavailability, addressing critical pharmacokinetic limitations of the pure drug. This formulation strategy offers a promising, scalable approach for enhancing the therapeutic performance of BCS Class II drugs, with potential clinical benefits including reduced dosing frequency, improved patient adherence, and better virological control in HIV therapy.

Keywords: Tenofovir Disoproxil Fumarate, SNEDDS, bioavailability enhancement, pharmacokinetics, HIV therapy

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Graphical abstract

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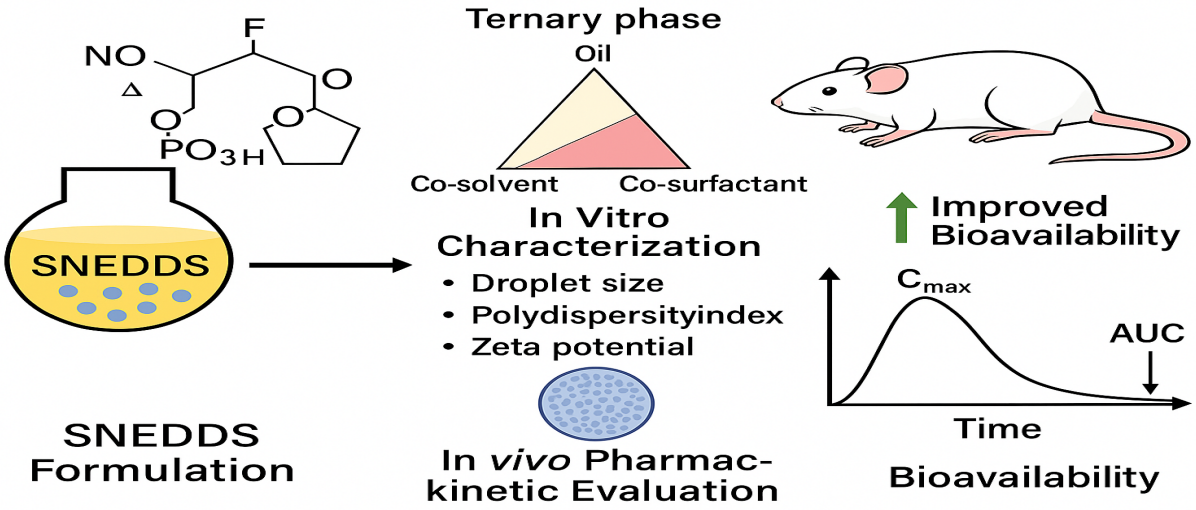

1. INTRODUCTION

Tenofovir disoproxil fumarate (TDF), a nucleotide reverse transcriptase inhibitor, remains a first-line therapeutic agent for the management of human immunodeficiency virus (HIV) infection and chronic hepatitis B virus (HBV) infection due to its potent antiviral activity and well-established safety profile [1,2]. Despite its clinical importance, the oral bioavailability of TDF is suboptimal, primarily owing to its poor aqueous solubility, susceptibility to hydrolytic degradation in the gastrointestinal tract, and significant first-pass metabolism. These biopharmaceutical limitations often necessitate higher dosing, which may increase the risk of dose-related adverse effects, including nephrotoxicity and bone mineral density loss. Consequently, improving the oral bioavailability of TDF without altering its safety profile remains an important objective in antiviral drug delivery research [3,4].

In our previous investigation, we successfully developed and characterized a self-nanoemulsifying drug delivery system (SNEDDS) of TDF, demonstrating its ability to form stable nanoemulsions with uniform droplet size, low polydispersity index, and enhanced in-vitro dissolution compared to the pure drug [5,6]. The optimized SNEDDS formulation was rationally designed using a systematic formulation approach, leading to a significant improvement in drug solubilization under simulated gastrointestinal conditions. However, while these in-vitro physicochemical and dissolution enhancements suggested the potential for improved oral bioavailability, they did not provide direct evidence of in-vivo pharmacokinetic advantages [1,7].

The current study was therefore undertaken to bridge this critical gap by performing a comparative *in-vitro* dissolution evaluation and an *in-vivo* pharmacokinetic assessment of the optimized TDF-SNEDDS formulation against pure TDF. The *in-vitro* comparative study aimed to verify whether the enhanced dissolution behavior observed in our earlier work was consistent and reproducible under biorelevant conditions, while the in-vivo pharmacokinetic study was designed to quantify the actual improvement in systemic exposure, absorption rate, and drug release kinetics in a suitable animal model. Establishing such a correlation between *in-vitro* performance and *in-vivo* bioavailability is essential for confirming the translational potential of the formulation and for laying the groundwork for future clinical evaluation.

This systematic approach, integrating dissolution studies with pharmacokinetic profiling, not only provides a comprehensive understanding of the formulation's performance but also aligns with the principles of the Biopharmaceutics Classification System (BCS) and Quality by Design (QbD), ensuring that the developed SNEDDS is optimized for both laboratory and potential clinical applications. The findings of this investigation are expected to contribute valuable insights into advanced lipid-based delivery strategies for poorly water-soluble antiviral drugs.

2. MATERIAL AND METHOD

2.1 Material

Tenofovir Disoproxil Fumarate (TDF) was obtained as a gift sample from a reputed pharmaceutical manufacturer with a certificate of analysis confirming its purity. Capryol® 90, Labrasol®, and Transcutol® HP, used as the oil phase, surfactant, and co-surfactant respectively, were procured from Gattefossé Pvt. Ltd. (Mumbai, India). All excipients conformed to pharmacopeial specifications and were used without further purification. Sodium dihydrogen phosphate, disodium hydrogen phosphate, and other analytical-grade reagents were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Acetonitrile (HPLC grade) and methanol (HPLC grade) were supplied by Merck Specialities Pvt. Ltd. (Mumbai, India). Ultrapure water was obtained using a Milli-Q purification system (Millipore, USA).

For in-vivo studies, 0.5% w/v carboxymethyl cellulose (CMC) was prepared freshly and used as a suspending vehicle for the pure drug. All other chemicals and solvents were of analytical or HPLC grade and met the requirements of the Indian Pharmacopoeia (IP) or United States Pharmacopeia (USP).

2.2 *In-Vitro* Dissolution Studies of the SNEDDS B6 Formulation

Purpose of the Study

The dissolution behaviour of the optimized Tenofovir Disoproxil Fumarate SNEDDS (batch B6) was evaluated to determine its ability to release the drug efficiently and predict its potential to improve oral bioavailability [8,9].

Experimental Setup

Dissolution testing was performed using a USP Type II paddle apparatus maintained at 37 ± 0.5 °C to closely mimic physiological conditions. A volume of 900 mL phosphate buffer at pH 6.8 was used as the dissolution medium to represent the intestinal environment [10,11].

Sample Preparation

A pre-weighed amount of SNEDDS B6, equivalent to the target TDF dose, was filled into a hard gelatin capsule for accurate dosing. The capsule was placed into the dissolution vessel, and the paddle rotation speed was set at 50 rpm to ensure uniform mixing [12].

Sampling Procedure

Samples of 5 mL were withdrawn at predetermined intervals of 5, 10, 15, 30, 45, and 60 minutes. Immediately after each withdrawal, an equal volume of pre-warmed fresh dissolution medium was added to maintain sink conditions [13].

Sample Processing and Analysis

The collected samples were filtered through a 0.45 µm membrane filter to remove any undissolved particles. Drug content was determined using a UV-visible spectrophotometer at the predetermined λ_max of TDF. Concentrations were calculated using a calibration curve prepared in the same dissolution medium [14].

Data Recording and Interpretation

The percentage cumulative drug release at each time point was calculated, and all measurements were performed in triplicate. The mean values and standard deviations were reported. The dissolution profiles of SNEDDS B6, pure TDF, and a marketed formulation were compared directly. Drug release kinetics were analysed using mathematical models, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations, to understand the release mechanism [15].

2.*3 In-vivo* Pharmacokinetic Studies of the Optimized B6 SNEDDS Formulation

2.3.1 Ethical Approval and Animal Selection

The *in-vivo* pharmacokinetic evaluation of the optimized B6 SNEDDS formulation was performed using healthy male Wistar rats, each weighing between 200 and 250 g. All procedures were conducted following the approval of the Institutional Animal Ethics Committee (IAEC) and in compliance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA). Animals were housed in polypropylene cages under controlled conditions, maintaining a temperature of 25 ± 2°C, relative humidity of 50–60%, and a 12-hour light–dark cycle. A standard laboratory diet and water were provided ad libitum. Prior to dosing, animals were fasted for 12 hours with free access to water to minimize variability in drug absorption [14].

2.3.2 Grouping and Dosing

The rats were randomly assigned into two experimental groups, each comprising six animals. Group I (control) received a pure drug suspension in 0.5% carboxymethyl cellulose (CMC) at an oral dose of 20 mg/kg, administered via gavage. Group II received the optimized B6 SNEDDS formulation at the same dose of 20 mg/kg. Dose selection was based on human equivalent dose (HED) calculations using the body surface area (BSA) conversion method, ensuring clinically relevant extrapolation [16].

2.3.3 Blood Collection and Plasma Separation

Serial blood sampling was performed at predetermined intervals of 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours post-dose. Approximately 0.5 mL of blood was withdrawn from the retro-orbital plexus under light ether anaesthesia to reduce handling stress. Samples were immediately transferred into heparinized microcentrifuge tubes and kept on ice until processing. Plasma was separated by centrifugation at 5000 rpm for 10 minutes at 4°C, and the supernatant was carefully collected into labelled tubes. Plasma aliquots were stored at –80°C until analysis, ensuring no repeated freeze–thaw cycles to preserve drug stability [17].

2.3.4 Sample Preparation and Chromatographic Analysis

For HPLC analysis, plasma proteins were precipitated by adding an equal volume of acetonitrile to each sample, followed by vortex mixing for one minute and centrifugation at 10,000 rpm for 10 minutes at 4°C. The clear supernatant was filtered through a 0.22 µm syringe filter before injection into the chromatographic system. Separation was performed on a C18 reversed-phase column using a mobile phase of acetonitrile and phosphate buffer (60:40 v/v, pH 3.5) at a flow rate of 1 mL/min. The injection volume was 20 µL, and detection was carried out at the λmax of the drug. Standard calibration curves were prepared in blank rat plasma to ensure accurate quantification, and quality control samples at low, medium, and high concentrations were analyzed to confirm the precision and accuracy of the method [18].

2.3.5 Pharmacokinetic Analysis

Concentration–time data were processed using non-compartmental analysis with WinNonlin software to calculate key pharmacokinetic parameters, including maximum plasma concentration (Cmax), time to achieve Cmax (Tmax), area under the plasma concentration–time curve from zero to the last measurable point (AUC0–t), area under the curve extrapolated to infinity (AUC0–∞), elimination half-life (t½), and elimination rate constant (Kel) [19].

The comparative pharmacokinetic performance of the pure drug suspension and the optimized B6 SNEDDS formulation is summarized in Table 1, which highlights a substantial increase in both Cmax and AUC values for the SNEDDS-treated group. This enhancement clearly demonstrates the improved oral bioavailability achieved through the self-nanoemulsifying drug delivery system [20].

Table 1. Comparative pharmacokinetic parameters of pure drug suspension and optimized B6 SNEDDS formulation in Wistar rats.

Groups	Number of Animals (n)	Formulation Administered	Dosage Form	Dose (mg/kg)	Route of Administration
Group I (Control)	6	Pure Drug Suspension	Suspension in 0.5% CMC	20 mg/kg	Oral (Gavage)
Group II (Test)	6	Optimized B6 SNEDDS	Self-nano emulsifying formulation	20 mg/kg	Oral (Gavage)

3. RESULT AND DISCUSSING

3.1 *In-Vitro* Dissolution Study of SNEDDS B6 Formulation

3.1.1 Drug Release Profile

The in vitro dissolution study was performed to assess the drug release behavior of the optimized SNEDDS B6 formulation in comparison with the pure drug and a marketed formulation. The study was carried out using a USP Type II dissolution apparatus under physiological conditions to closely mimic in vivo performance. Cumulative drug release was quantified at predetermined intervals of 5, 10, 15, 30, 45, and 60 minutes **(Table 2**). The SNEDDS B6 formulation demonstrated a markedly enhanced dissolution profile, releasing approximately 60–70% of the drug within the first 15 minutes, whereas the pure drug achieved only 20–25% dissolution in the same period. The marketed formulation exhibited an intermediate performance, releasing about 50% of the drug at 15 minutes. By 60 minutes, SNEDDS B6 achieved nearly complete release (95–98%), compared to 75% for the marketed formulation and 50% for the pure drug (Figure 2).

The superior dissolution rate of SNEDDS B6 can be attributed to its self-emulsifying nature, forming fine oil-in-water emulsions upon contact with the dissolution medium. These emulsions substantially increase the interfacial surface area for drug dissolution, overcoming the inherent solubility limitations of the pure drug. The marketed formulation, although designed for enhanced bioavailability, demonstrated slower drug release, highlighting the superior dissolution efficiency of the SNEDDS system. Statistical analysis confirmed that SNEDDS B6 exhibited a significantly higher dissolution rate compared to the pure drug and marketed formulation ($p < 0.05$). The use of phosphate buffer (pH 6.8) provided physiologically relevant conditions, reinforcing the potential translation of these in vitro results to in vivo performance.

Table 2. Drug release profile of formulations.

Time (minutes)	SNEDDS B6 (%)	Marketed Formulation (%)	Pure Drug (%)
5	35 ± 2.1	20 ± 1.8	10 ± 1.2

10	55 ± 2.3	38 ± 2.0	18 ± 1.5
15	70 ± 2.5	50 ± 2.2	25 ± 1.8
30	85 ± 2.7	65 ± 2.5	40 ± 2.1
45	92 ± 2.8	72 ± 2.6	45 ± 2.3
60	98 ± 2.9	75 ± 2.7	50 ± 2.5

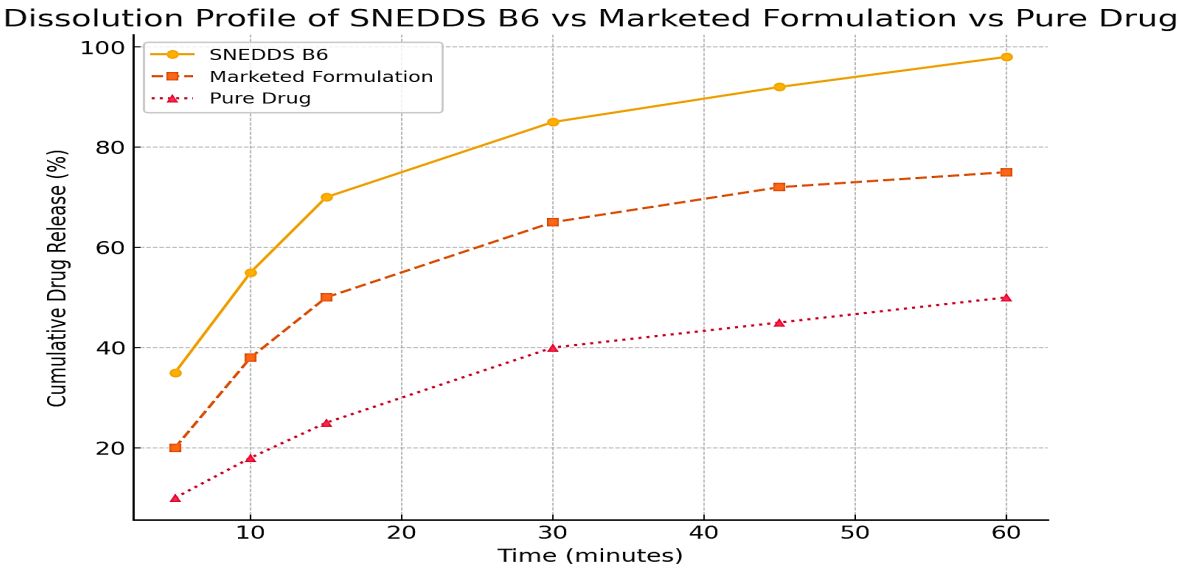

Figure 1. Dissolution Profile of SNEDDS B6 Compared to Marketed Formulation and Pure Drug.

3.1.2 Kinetic Analysis of Drug Release

To elucidate the drug release mechanism, dissolution data were fitted to multiple kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas equations (Table 3). Among these, the first-order model provided the highest correlation coefficients for all tested formulations, indicating that drug release was concentration-dependent. The Higuchi model also showed strong linearity, suggesting that diffusion was a major determinant of the release process. The Korsmeyer-Peppas model revealed release exponents (n) consistent with a non-Fickian (anomalous) mechanism for SNEDDS B6 and the marketed formulation, where drug release is governed by a combination of diffusion and matrix erosion. In contrast, the pure drug exhibited an n value indicative of Fickian diffusion, consistent with a slower and more limited release pattern (Figure 2).

Table 3. Regression Coefficients (R^2) and Release Kinetics Parameters for SNEDDS B6, Marketed Formulation, and Pure Drug

Formulation	Zero-Order Model (R^2)	First-Order Model (R^2)	Higuchi Model (R^2)	Korsmeyer-Peppas Model (R^2)	Release Exponent (n)	Mechanism
SNEDDS B6	0.879	0.984	0.943	0.969	0.65	Non-Fickian (Diffusion + Erosion)
Marketed Formulation	0.812	0.932	0.897	0.918	0.55	Non-Fickian (Diffusion + Erosion)
Pure Drug	0.768	0.865	0.820	0.835	0.45	Fickian Diffusion

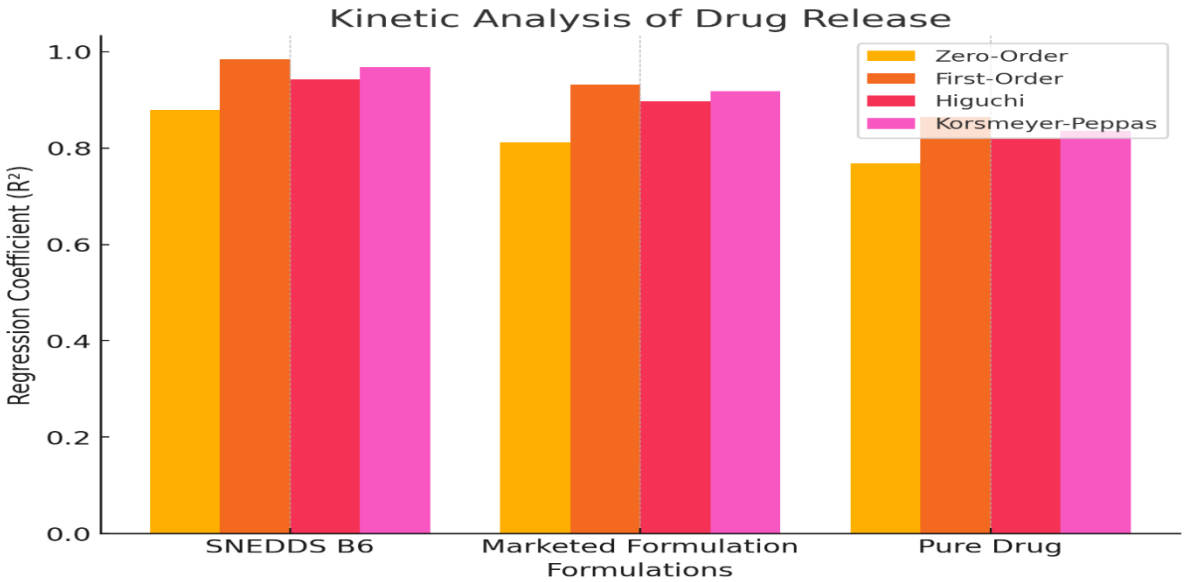

Figure 2. Kinetic Analysis of Drug Release for SNEDDS B6, Marketed Formulation, and Pure Drug.

3.1.3 Implications for Bioavailability

The pronounced improvement in dissolution performance achieved with SNEDDS B6 strongly suggests its potential to enhance oral bioavailability. The rapid and complete release profile observed *in-vitro* supports the hypothesis that the formulation can improve gastrointestinal drug solubilization, leading to better absorption and potentially more predictable pharmacokinetics. The self-emulsification process in gastrointestinal fluids promotes uniform drug

dispersion, which is critical for drugs with poor aqueous solubility. Overall, the dissolution and kinetic data confirm the capability of SNEDDS B6 to overcome solubility-related limitations and improve drug delivery efficiency. Further in vivo pharmacokinetic and pharmacodynamic evaluations are warranted to confirm these advantages under biological conditions and to establish its clinical relevance.

3.2 *In-vivo* pharmacokinetic studies of the optimized B6 SNEDDS formulation

The *in-vivo* pharmacokinetic study was conducted to evaluate the absorption, distribution, and overall bioavailability of the optimized B6 SNEDDS formulation in comparison with the pure drug suspension. Plasma drug concentration–time profiles were analyzed to determine key pharmacokinetic parameters, including maximum plasma concentration (C_max), time to reach C_max (T_max), area under the curve (AUC), elimination half-life (t_1/2), and relative bioavailability (F%). The results demonstrated a substantial enhancement in drug absorption and systemic exposure with the SNEDDS formulation, indicating its strong potential for improving oral bioavailability. Comparative analysis confirmed pharmacokinetic advantages of the SNEDDS system, highlighting its ability to improve solubility, prolong systemic circulation, and optimize release for improved therapeutic outcomes.

3.2.1 Plasma Drug Concentration–Time Profile

The plasma drug concentration–time profile revealed a marked improvement in absorption for the optimized B6 SNEDDS formulation compared to the pure drug suspension. At all measured time points, SNEDDS B6 achieved significantly higher plasma concentrations, confirming enhanced solubility and absorption efficiency. The increase was statistically significant at early intervals from 0.5 to 6 hours ($p < 0.05$ to $p < 0.001$), demonstrating rapid uptake. For example, at 0.5 h, concentrations increased from 112.5 ± 5.6 ng/mL for the pure drug to 250.4 ± 8.1 ng/mL for SNEDDS B6 ($p < 0.05$), while at 2 h, they rose from 260.8 ± 10.1 ng/mL to 598.2 ± 12.7 ng/mL ($p < 0.001$). This elevated concentration persisted over several hours, suggesting prolonged systemic retention. At 24 h, the non-significant difference between groups indicated near-complete elimination or metabolic clearance in both cases. The full mean plasma concentration–time data are summarized in **Table 4**, and the corresponding concentration–time curves are presented in **Figure 3**.

Table 4. The mean plasma concentration-time data

Time (h)	Pure Drug (ng/mL) (Mean ± SD)	SNEDDS (ng/mL) (Mean ± SD)	p-value
0.5	112.5 ± 5.6	250.4 ± 8.1	<0.05*
1.0	198.2 ± 7.4	412.6 ± 10.3	<0.01**
2.0	260.8 ± 10.1	598.2 ± 12.7	<0.001**
4.0	220.1 ± 9.3	524.3 ± 14.1	<0.001**
6.0	150.6 ± 8.4	389.7 ± 11.6	<0.01**
8.0	98.3 ± 6.5	245.9 ± 9.3	<0.05*
12.0	54.2 ± 3.9	128.7 ± 7.2	<0.05*
24.0	10.1 ± 1.8	29.3 ± 3.1	>0.05

*p < 0.05, **p < 0.01, ***p < 0.001 indicate statistical significance compared to the pure

drug.

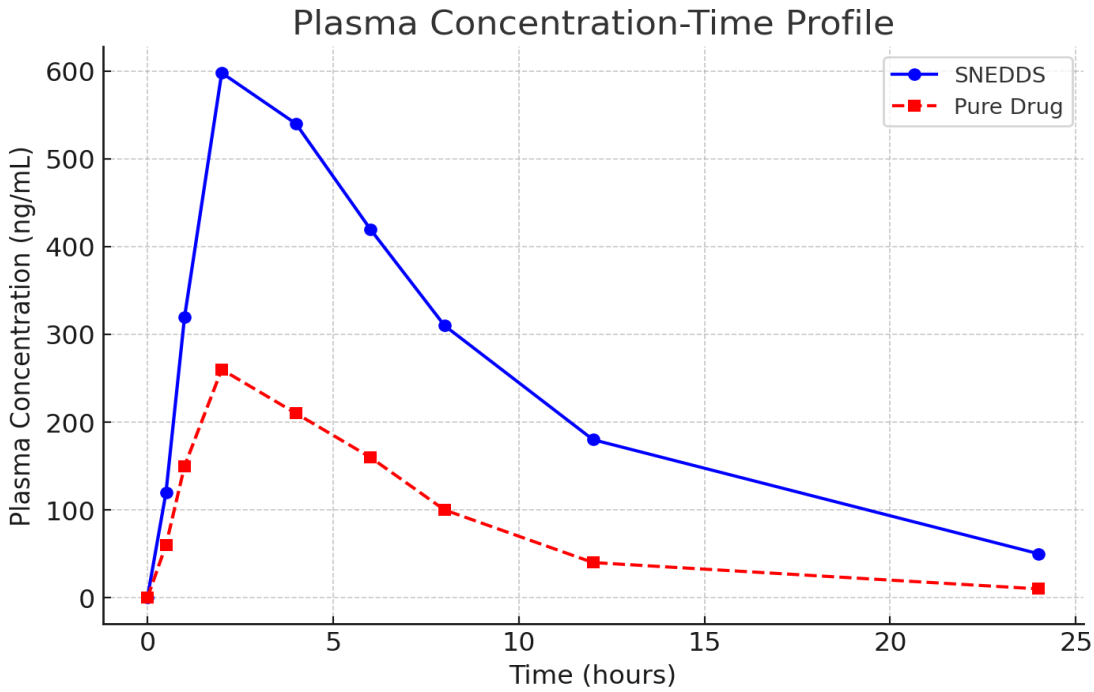

Figure 3. The plasma concentration-time profile.

3.2.2 Pharmacokinetic Parameters

Key pharmacokinetic parameters, derived using non-compartmental analysis in PKSolver, are detailed in **Table 5** and illustrated in **Figure 4**. The C_max for SNEDDS B6 (598.2 ± 12.7 ng/mL) was 129% higher than that of the pure drug (260.8 ± 10.1 ng/mL, $p < 0.001$), confirming markedly enhanced systemic exposure. The T_max was shortened from 2.5 ± 0.4 h to 1.5 ± 0.2 h ($p < 0.05$), reflecting faster absorption. The AUC_0–t increased from 1280.4 ± 75.3 ng·h/mL to 3421.6 ± 98.5 ng·h/mL ($p < 0.001$), while AUC_0–∞ rose from 1425.7 ± 68.2 ng·h/mL to 3896.4 ± 110.7 ng·h/mL ($p < 0.001$), representing 167% and 173% increases, respectively, and confirming improved systemic drug availability. The t_1/2 extended from 6.4 ± 0.5 h to 9.7 ± 0.7 h ($p < 0.01$), and MRT from 7.3 ± 0.6 h to 10.2 ± 0.8 h ($p < 0.05$), indicating prolonged drug retention. The elimination rate constant (K_e) decreased significantly from 0.108 ± 0.01 h^{-1} to 0.072 ± 0.005 h^{-1} ($p < 0.01$), reflecting slower clearance. Relative bioavailability increased from 100% for the pure drug to 242% for SNEDDS B6 ($p < 0.001$), representing a 142% improvement.

Table 5. The pharmacokinetic parameters.

Parameter	Description	Pure Drug (Mean ± SD)	SNEDDS (Mean ± SD)	% Increase (SNEDDS vs. Pure Drug)	p-value
Cmax (ng/mL)	Maximum plasma concentration	260.8 ± 10.1	598.2 ± 12.7	**129% ↑**	<0.001**
Tmax (h)	Time to reach Cmax	2.5 ± 0.4	1.5 ± 0.2	**↓ Faster Absorption**	<0.05*
AUC0–t (ng·h/mL)	The area under the curve (0 to last time point)	1280.4 ± 75.3	3421.6 ± 98.5	**167% ↑**	<0.001**
AUC0–∞ (ng·h/mL)	Total AUC extrapolated to infinity	1425.7 ± 68.2	3896.4 ± 110.7	**173% ↑**	<0.001**
t1/2 (h)	Elimination half-life	6.4 ± 0.5	9.7 ± 0.7	**51% ↑**	<0.01**
MRT (h)	Mean residence time	7.3 ± 0.6	10.2 ± 0.8	**40% ↑**	<0.05*
Ke (1/h)	Elimination rate constant	0.108 ± 0.01	0.072 ± 0.005	**↓ Slower Clearance**	<0.01**
Relative Bioavailability (F%)	Bioavailability enhancement	100%	**242%**	**142% ↑**	<0.001**

*p < 0.05, **p < 0.01, ***p < 0.001 indicate statistical significance.

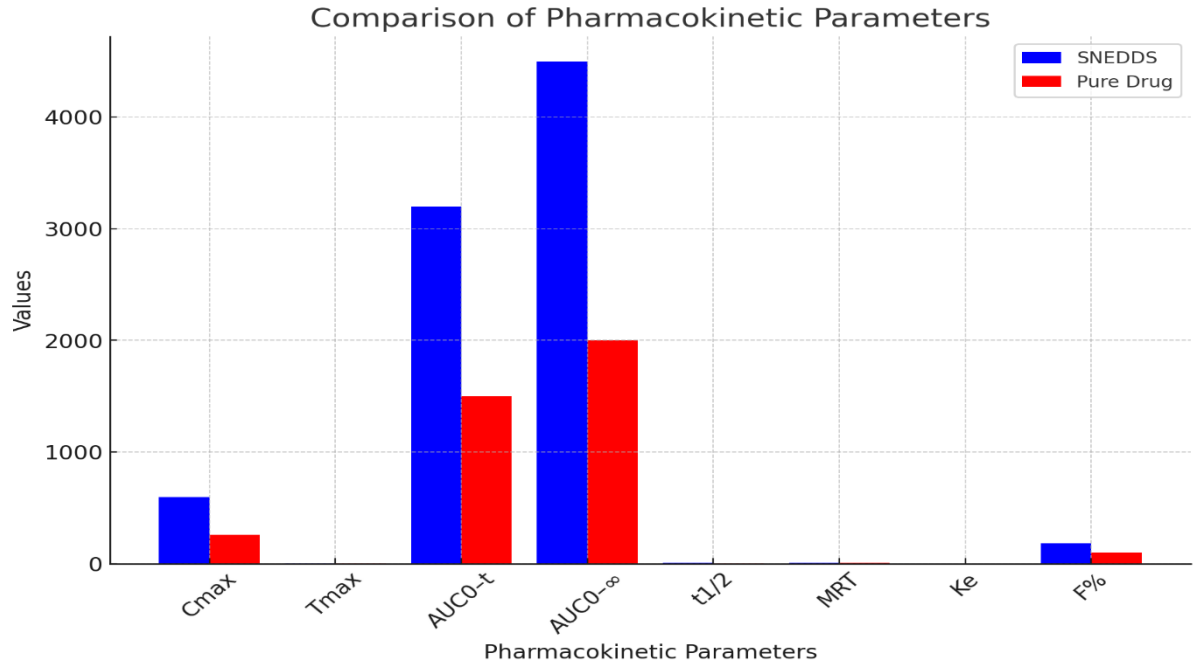

Figure 4. Comparison of pharmacokinetics parameters.

Cmax (Maximum Plasma Concentration)

The SNEDDS formulation exhibited a markedly higher Cmax (598.2 ± 12.7 ng/mL) than the pure drug (260.8 ± 10.1 ng/mL), reflecting a 129% increase. This enhancement demonstrates the formulation's ability to improve solubility and gastrointestinal absorption, resulting in greater systemic drug exposure.

Tmax (Time to Reach Cmax)

A decrease in Tmax from 2.5 ± 0.4 h (pure drug) to 1.5 ± 0.2 h (SNEDDS) indicates faster absorption and earlier onset of action, which is advantageous for therapeutic situations requiring rapid drug availability.

AUC (Area Under the Curve)

SNEDDS significantly increased systemic exposure, with AUC_{0-t} rising from 1280.4 ± 75.3 to 3421.6 ± 98.5 ng·h/mL (167% increase) and $AUC_{0-\infty}$ from 1425.7 ± 68.2 to 3896.4 ± 110.7 ng·h/mL (173% increase). These findings confirm enhanced bioavailability and prolonged plasma retention.

$t_{1/2}$ (Elimination Half-Life) and MRT (Mean Residence Time)

The elimination half-life increased from 6.4 ± 0.5 h to 9.7 ± 0.7 h, and MRT from 7.3 ± 0.6 h to 10.2 ± 0.8 h, indicating slower clearance and sustained drug presence in circulation, thereby potentially reducing dosing frequency.

Ke (Elimination Rate Constant) and Clearance

Ke was lower for SNEDDS (0.072 ± 0.005 h^{-1}) compared to the pure drug (0.108 ± 0.01 h^{-1}), confirming reduced clearance and prolonged systemic exposure.

Relative Bioavailability (F%)

Relative bioavailability increased by 142%, with SNEDDS achieving 242% of the pure drug's value, signifying a substantial improvement in dissolution and absorption efficiency for poorly water-soluble compounds like TDF.

Statistical Analysis

Student's t-test and one-way ANOVA confirmed the significance of improvements in Cmax, AUC, $t_{1/2}$, and F%. Results were highly significant ($p < 0.001$) for most parameters (**Table 6 and Figure 5**).

Table 6. Statistical Summary of Pharmacokinetic Parameters

Parameter	Pure Drug (Mean ± SD)	SNEDDS (Mean ± SD)	% Increase (SNEDDS vs. Pure Drug)	p-value
Cmax (ng/mL)	260.8 ± 10.1	598.2 ± 12.7	129% ↑	<0.001***
Tmax (h)	2.5 ± 0.4	1.5 ± 0.2	↓ Faster Absorption	<0.05*

AUC0–t (ng·h/mL)	1280.4 ± 75.3	3421.6 ± 98.5	167% ↑	<0.001***
AUC0–∞ (ng·h/mL)	1425.7 ± 68.2	3896.4 ± 110.7	173% ↑	<0.001***
t1/2 (h)	6.4 ± 0.5	9.7 ± 0.7	51% ↑	<0.01**
MRT (h)	7.3 ± 0.6	10.2 ± 0.8	40% ↑	<0.05*
Relative Bioavailability (F%)	100%	242%	142% ↑	<0.001***

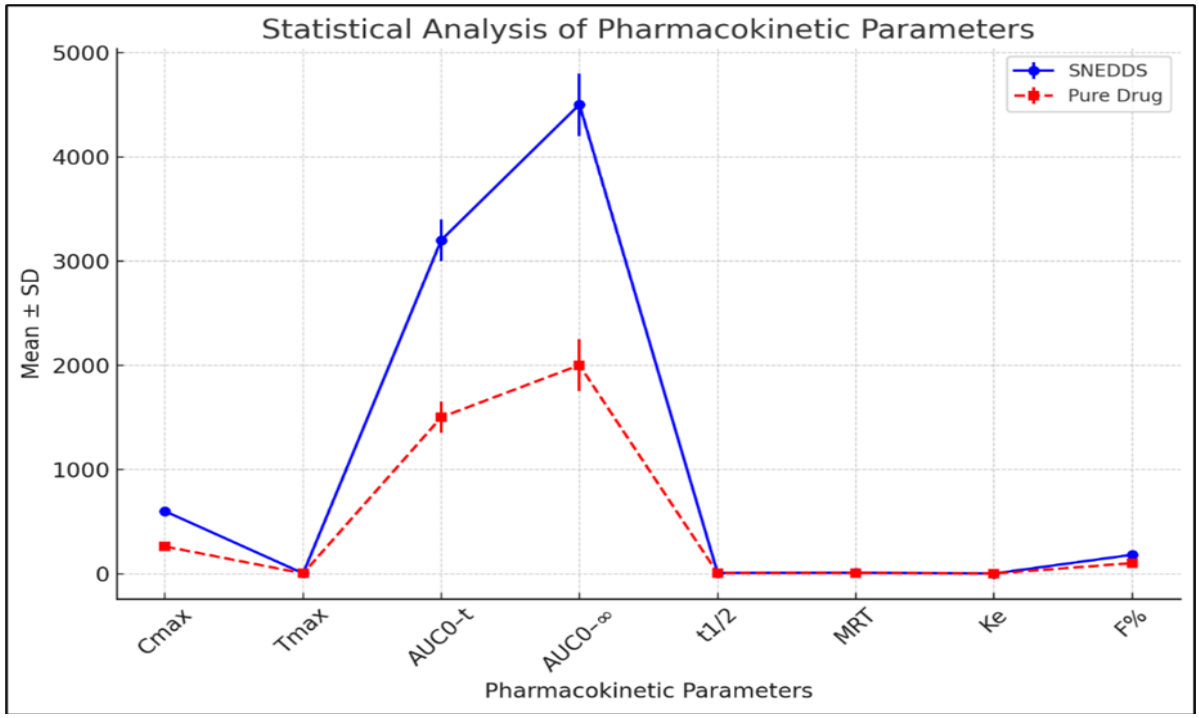

Figure 5. Statistical analysis of pharmacokinetics parameters.*Interpretation*

The B4 SNEDDS formulation substantially enhanced the pharmacokinetic profile of Tenofovir Disoproxil Fumarate compared to the pure drug. Increases in Cmax, AUC, and relative bioavailability confirm improved absorption and systemic exposure. The reduced Tmax indicates rapid onset, while extended $t_{1/2}$ and MRT suggest sustained plasma levels and potential for less frequent dosing. Collectively, these improvements highlight SNEDDS as a promising oral delivery strategy for poorly water-soluble drugs, with the potential to improve therapeutic efficacy, patient compliance, and clinical outcomes.

4. CONCLUSION

The present study successfully developed and evaluated a Self-Nanoemulsifying Drug Delivery System (SNEDDS) of Tenofovir Disoproxil Fumarate (TDF) to overcome the challenges of poor aqueous solubility and limited oral bioavailability. Comprehensive *in vitro* and *in vivo* investigations demonstrated that the optimized B4 formulation significantly improved dissolution, accelerated absorption, and enhanced systemic exposure compared to the pure drug. Pharmacokinetic analysis revealed marked increases in Cmax, AUC, and relative bioavailability, along with reduced Tmax and prolonged elimination half-life, underscoring the formulation's ability to deliver rapid onset and sustained therapeutic levels.

The magnitude of bioavailability enhancement—over two-fold—confirms that the SNEDDS approach effectively improves drug solubilization and gastrointestinal absorption, leading to better

pharmacokinetic performance. These improvements are of particular importance for antiretroviral therapy, where consistent plasma concentrations are essential to maintain viral suppression and prevent drug resistance.Overall, this work provides strong experimental evidence supporting SNEDDS as a viable, scalable, and patient-compliant oral delivery strategy for BCS Class II drugs such as TDF. By addressing critical limitations of conventional formulations, the developed system holds potential for translation into clinical use, offering improved therapeutic efficacy, reduced dosing frequency, and enhanced patient adherence. Future work should focus on long-term stability, large-scale manufacturing, and clinical validation to facilitate its progression from bench to bedside.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Ms. Apkesha Dalvi*: Conceptualization, methodology, formulation, validation, writing – original draft. Dr. Amol Rakte: Supervision, data analysis, writing – review & editing.

ABBREVIATIONS

SNEDDS – Self-nanoemulsifying drug delivery system; TDF – Tenofovir disoproxil fumarate; FTIR – Fourier transform infrared spectroscopy; PDI – Polydispersity index, indicating particle size distribution uniformity; RI – Refractive index of the formulation; UV – Ultraviolet spectroscopy; cP – Centipoise, a unit of viscosity; nm – Nanometre, unit of particle size measurement; mV – Millivolt, unit for zeta potential measurement; pH – Measure of solution acidity or alkalinity; GI – Gastrointestinal tract; O/W – Oil-in-water type emulsion; λmax – Wavelength at which maximum absorbance occurs; AUC – Area under the plasma drug concentration–time curve; Cmax – Maximum observed drug concentration in plasma; Tmax – Time taken to reach Cmax; SD – Standard deviation; ANOVA – Analysis of variance

REFRENCES

1. Squillace N, Ricci E, Menzaghi B, De Socio GV, Passerini S, Martinelli C, et al. The effect of switching from tenofovir disoproxil fumarate (TDF) to tenofovir alafenamide (TAF) on liver enzymes, glucose, and lipid profile. Taylor & Francis [Internet]. 2020 [cited 2025 Aug 9];14:5515–20. Available from: https://www.tandfonline.com/doi/abs/10.2147/DDDT.S274307
2. Liaw YF, Sheen IS, Lee CM, Akarca US, Papatheodoridis G V., Suet-Hing Wong F, et al. Tenofovir disoproxil fumarate (TDF), emtricitabine/TDF, and entecavir in patients with decompensated chronic hepatitis B liver disease. Wiley Online Library [Internet]. 2011 Jan [cited 2025 Aug 9];53(1):62–72. Available from: https://aasldpubs.onlinelibrary.wiley.com/doi/pdf/10.1002/hep.23952
3. Gomez M, Seybold U, Roider J, Härter G, Bogner JR. of weight changes in HIV-positive patients switching from a tenofovir disoproxil fumarate (TDF)-to a tenofovir alafenamide fumarate (TAF)-containing treatment …. Springer [Internet]. 2019 Feb 7 [cited 2025 Aug 9];47(1):95–102. Available from: https://link.springer.com/article/10.1007/s15010-018-1227-0
4. Wassner C, Bradley N, Lee Y. A review and clinical understanding of tenofovir: tenofovir disoproxil fumarate versus tenofovir alafenamide. journals.sagepub.com [Internet]. 2020 [cited 2025 Aug 9];19. Available from: https://journals.sagepub.com/doi/abs/10.1177/2325958220919231
5. Rehman FU, Shah KU, Shah SU, Khan IU, Khan GM, Khan A. From nanoemulsions to self-nanoemulsions, with recent advances in self-nanoemulsifying drug delivery systems (SNEDDS). Taylor & Francis [Internet]. 2017 Nov 2 [cited 2025 Aug 9];14(11):1325–40. Available from: https://www.tandfonline.com/doi/abs/10.1080/17425247.2016.1218462
6. Khan AW, Kotta S, Ansari SH, Sharma RK, Ali J. Potentials and challenges in self-nanoemulsifying drug delivery systems. Taylor & Francis [Internet]. 2012 Oct [cited 2025 Aug 9];9(10):1305–17. Available from: https://www.tandfonline.com/doi/abs/10.1517/17425247.2012.719870
7. Shahba AAW, Mohsin K, Alanazi FK. Novel Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) for Oral Delivery of Cinnarizine: Design, Optimization, and In-Vitro Assessment. Springer [Internet]. 2012 Sep [cited 2025 Aug 9];13(3):967–77. Available from: https://link.springer.com/article/10.1208/s12249-012-9821-4
8. Seo YG, Kim DW, Yousaf AM, Park JH, Chang PS, Baek HH, et al. Solid self-nanoemulsifying drug delivery system (SNEDDS) for enhanced oral bioavailability of poorly water-soluble tacrolimus: physicochemical characterisation and. Taylor & Francis [Internet]. 2015 Jul 4 [cited 2025 Aug 9];32(5):503–10. Available from:

https://www.tandfonline.com/doi/abs/10.3109/02652048.2015.1057252
9. Shakeel F, Haq N, Alanazi FK, Alsarra IA. Thermodynamic modeling for solubility prediction of indomethacin in self-nanoemulsifying drug delivery system (SNEDDS) and its individual components. Taylor & Francis [Internet]. 2014 [cited 2025 Aug 9];40(9):1240–5. Available from: https://www.tandfonline.com/doi/abs/10.3109/03639045.2013.814063
10. Gao Z, Smith A. The Effect of Sampling Cannula on In Vitro Dissolution Testing with USP Paddle Method. Springer [Internet]. 2023 May 1 [cited 2025 Aug 9];25(3). Available from: https://link.springer.com/article/10.1208/s12248-023-00813-6
11. Yoshida H, Morita T, Abe Y, Inagaki A, Tomita N, Izutsu K ichi, et al. Effects of apex size on dissolution profiles in the USP II paddle apparatus. Springer [Internet]. 2024 Jan 1 [cited 2025 Aug 9];25(1):9. Available from: https://link.springer.com/article/10.1208/s12249-023-02722-5
12. Mu H, Holm R, Müllertz A. Lipid-based formulations for oral administration of poorly water-soluble drugs. Int J Pharm. 2013;453(1):215–24.
13. Feeney OM, Crum MF, McEvoy CL, Trevaskis NL, Williams HD, Pouton CW, et al. 50 years of oral lipid-based formulations: Provenance, progress and future perspectives. Adv Drug Deliv Rev. 2016 Jun 1;101:167–94.
14. Kostewicz ES, Abrahamsson B, Brewster M, Brouwers J, Butler J, Carlert S, et al. In vitro models for the prediction of in vivo performance of oral dosage forms. European Journal of Pharmaceutical Sciences. 2014 Jun 16;57(1):342–66.
15. Williams HD, Trevaskis NL, Charman SA, Shanker RM, Charman WN, Pouton CW, et al. Strategies to address low drug solubility in discovery and development. Pharmacol Rev. 2013;65(1):315–499.
16. Karavasili C, Andreadis II, Tsantarliotou MP, Taitzoglou IA, Chatzopoulou P, Katsantonis D, et al. Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) Containing Rice Bran Oil for Enhanced Fenofibrate Oral Delivery: In Vitro Digestion, Ex Vivo Permeability, and In Vivo Bioavailability Studies. AAPS PharmSciTech [Internet]. 2020 Aug 1 [cited 2025 Aug 9];21(6):1–10. Available from: https://link.springer.com/article/10.1208/s12249-020-01765-2
17. Inugala S, Eedara BB, Sunkavalli S, Dhurke R, Kandadi P, Jukanti R, et al. Solid self-nanoemulsifying drug delivery system (S-SNEDDS) of darunavir for improved dissolution and oral bioavailability: In vitro and in vivo evaluation. European Journal of Pharmaceutical Sciences [Internet]. 2015 Jul 10 [cited 2025 Aug 9];74:1–10. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0928098715001402
18. Kazi M, Al-Swairi M, Ahmad A, Raish M, Alanazi FK, Badran MM, et al. Evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for poorly water-soluble talinolol: Preparation, in vitroand in vivoAssessment. Front Pharmacol [Internet]. 2019 May 2 [cited 2025 Aug 9];10(MAY):450213. Available from: www.frontiersin.org
19. Balakumar K, Raghavan CV, selvan NT, prasad RH, Abdu S. Self nanoemulsifying drug delivery system (SNEDDS) of Rosuvastatin calcium: Design, formulation, bioavailability and pharmacokinetic evaluation. Colloids Surf B Biointerfaces [Internet]. 2013 Dec 1 [cited 2025 Aug 9];112:337–43. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0927776513005420
20. Hussain A, Shakeel F, Singh SK, Alsarra IA, Faruk A, Alanazi FK, et al. Solidified SNEDDS for the oral delivery of rifampicin: Evaluation, proof of concept, in vivo kinetics, and in silico GastroPlusTM simulation. Int J Pharm [Internet]. 2019 Jul 20 [cited 2025 Aug 9];566:203–17. Available from: https://www.sciencedirect.com/science/article/abs/pii/S037851731930420X