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Enhanced Bioavailability of Empagliflozin using Self-nanoemulsifying Drug Delivery System (SNEDDS): In vivo Pharmacokinetic Evaluation in rats

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Abstract

Empagliflozin (EFZ), a sodium-glucose co-transporter 2 (SGLT2) inhibitor used to treat type 2 diabetes, faces challenges with poor solubility and bioavailability. This study aimed to evaluate a Self-Nanoemulsifying Drug Delivery System (SNEDDS), specifically SN1C, for enhancing EFZ's dissolution rate and pharmacokinetics compared to pure EFZ powder and a marketed tablet (MKT). We assessed dissolution profiles in vitro and conducted pharmacokinetic studies in rats following oral administration of the three formulations. Plasma samples were analyzed using LC-MS/MS, and pharmacokinetic parameters were determined via non-compartmental analysis. SN1C demonstrated the highest dissolution rate in vitro. In vivo, SN1C achieved a significantly higher maximum plasma concentration (Cmax) and area under the curve (AUC(inf)) than EFZ and MKT, with approximately 1.5-fold higher Cmax and a significantly greater AUC(inf) ($p < 0.05$). Tmax and half-life (t1/2) were similar across all formulations, indicating that SNEDDS did not negatively impact these parameters. The results suggest that SN1C substantially improves the bioavailability of EFZ, highlighting the efficacy of SNEDDS technology in enhancing the oral delivery and therapeutic effectiveness of poorly soluble drugs like EFZ.

Keywords: Empagliflozin; Pharmacokinetics; SNEDDS; LC-MS/MS; Dissolution; Bioavailability.

Introduction

Empagliflozin (EFZ) is a highly effective sodium-glucose co-transporter 2 (SGLT2) inhibitor used in the treatment of type 2 diabetes mellitus (T2DM). Despite its therapeutic efficacy, EFZ suffers from poor solubility and limited bioavailability, which can restrict its clinical effectiveness (Gupta et al., 2013). To overcome these challenges, various drug delivery systems have been explored to enhance the solubility and absorption of poorly soluble drugs [1]. Self-nanoemulsifying drug delivery systems (SNEDDS) represent a promising approach for improving the oral bioavailability of lipophilic drugs. SNEDDS are isotropic mixtures of oils, surfactants, and co-surfactants that spontaneously form nano-sized emulsions upon contact with gastrointestinal fluids [2]. This nanoemulsion formation leads to enhanced drug solubilization and improved permeation across the intestinal epithelium, thus increasing the drug's bioavailability [3,4]. In this study, we evaluated the potential of a SNEDDS formulation (SN1C) for improving the pharmacokinetic profile of EFZ compared to its pure form and a marketed tablet formulation (MKT).

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We aimed to compare the dissolution profiles and in vivo pharmacokinetics of EFZ administered as SNEDDS, pure powder, and the marketed tablet in rats. The results of this evaluation are expected to provide insights into the advantages of SNEDDS technology in enhancing the bioavailability of EFZ.

Material and Methods

Materials

Empagliflozin was procured from Cipla Limited, Vikhroli, India. Other chemicals and reagents used were of analytical reagent grade.

Methodology

In vivo pharmacokinetic (PK) studies were performed in rats to compare the rate and extent of absorption of the best formulation with the pure drug and marketed formulation when administered orally.

Selection of Best Performing Formulation

Based on the results, the best formulations from Solid Dispersions (SD2), Liquid SNEDDS (SN1C), and Solid SNEDDS (S-SN1C-A200) were compared for their dissolution profiles. The formulation with the highest dissolution rate was selected for the in vivo PK studies in rats.

LC-MS/MS Method Development for Estimating Empagliflozin in Bulk and in Formulation

The LC-MS/MS method for the quantitative analysis of Empagliflozin in a lower volume of rat plasma (0.1 mL) was developed and applied to the PK study in rats (Kobuchi et al. 2016). Empagliflozin (EMP) was used as the internal standard (IS).

Preparation of Calibration Curve

Instrument and LC-MS/MS Conditions

The LC-MS/MS system consisted of an API-4500 system with Analyst 1.7 equipped with Nexera X2 LC. The method utilized a binary gradient mobile phase consisting of 10 mM ammonium acetate in water (Pump A) and 0.1% formic acid in acetonitrile (Pump B) with a flow rate of 600 µL/min. The analytical column was a Synergi Polar (75 mm × 2.0 mm, 4 µm size) maintained at 40 °C. The mass spectrometer used a selected ion monitoring method in the positive ion mode.

Table 1: Chromatographic Conditions

Parameter	Details
Mass Spectra	API-4500 (LC-MS/MS) system with Analyst 1.7
HPLC	Nexera X2 LC
Column	Synergi Polar, 75 × 2.0 mm, 4 µm
Mode	ESI Positive Mode
Flow Rate	600 µL/min
Run Time	3 min
Injection Volume	5 µL
Column Oven Temperature	40 °C
Autosampler Temperature	8 °C
Scan Mode	Positive mode

Table 2: Gradient Conditions:

Time (min)	% A	% B
0	98	2
0.01	98	2
2	2	98
2.5	2	98
2.6	98	2
3	98	2

Table 3: Source Parameters

Parameter	Value
Curtain Gas	40
CAD Gas	10
GS 1	50
GS 2	45
Nebulizer Current	5
Temperature	550 °C
Interface Heater	On

Preparation of Samples

Plasma calibration standards were prepared by serial dilution over the concentration range of 5–2000 ng/mL using methanol as a solvent. Quality control (QC) samples were prepared at concentrations of 50 (low) and 1000 (high) ng/mL. From the stock solutions, 20 µL of plasma standards, QC standards, and study samples were transferred to a 1.1 mL 96-well plate. To these samples, 120 µL of acetonitrile containing IS (EMP) was added. All samples were vortexed for 5 minutes and centrifuged at 4000 rpm for 10 minutes. 100 µL of the supernatant was transferred and injected into the LC-MS/MS system.

Table 4: Compound-Dependent Parameters:

Name	Q1 (m/z)	Q3 (m/z)	Dwell Time (msec)	DP (V)	EP (V)	CE (V)	CXP (V)
Empagliflozin	468.2	397.3	50	85	10	23	11

In vivo PK Studies

The in vivo PK studies of Empagliflozin and its formulations were approved by the Institutional Animal Ethics Committee (IAEC) of Deshpande Laboratories, Bhopal, Madhya Pradesh. The studies were conducted on male Sprague Dawley rats, aged 7-9 weeks, with a body weight of 250-300 g at the time of dosing. The animals were acclimatized for seven days, during which they were habituated to the cages, and their health status was monitored. Animals were uniquely labeled on their tails using a permanent marker (e.g., Animal #1, #2). The animals were allowed free access to feed and water ad libitum.

Study Design

Three dosing groups were formed, each consisting of 3 animals. The first group was administered Empagliflozin suspension, the second group received the marketed tablet formulation (MKT) suspension, and the third group was given the SN1C formulation dispersed in distilled water.

Table 5: Study Design and Dosing Details:

Parameter	Group 1	Group 2	Group 3
Compound Details (Code)	Pure Empagliflozin	Marketed Tablet Formulation (MKT)	Liquid SNEDDS Formulation (SN1C)
Formulation Details	15 mg powder dissolved in 15 mL of 0.5% CMC in water	One Tablet dissolved in 10 mL of 0.5% CMC in water	10 mL of preconcentrate dissolved in 50 mL of distilled water
Physical Appearance	Suspension	Suspension	Nanoemulsion
Route of Administration	Per Oral	Per Oral	Per Oral
Dose (mg/kg)	10	10	10
Dosing Volume (mL/kg)	10	10	10
Dose Concentration (mg/mL)	1	1	1
Group No.	1	2	3
No. of Animals/Group	3	3	3
Species	Rat	Rat	Rat
Strain	Sprague Dawley	Sprague Dawley	Sprague Dawley
Sex	Male	Male	Male
Fed/Fasted	Overnight fasted	Overnight fasted	Overnight fasted
Age	~7-8 weeks	~7-8 weeks	~7-8 weeks
Body Weight	~250-300 g	~250-300 g	~250-300 g
Sample Type	Plasma	Plasma	Plasma
Blood Collection Method	Through Jugular vein cannula	Through Jugular vein cannula	Through Jugular vein cannula
Anticoagulant Used	0.2% K2 EDTA	0.2% K2 EDTA	0.2% K2 EDTA

Dosing Procedure

The exact dose was administered based on the body weight (10 mg/kg body weight) of the animals on the day of dosing. The animals were fasted overnight before the day of dosing. Each animal received a single oral dose via a gavage needle.

Blood samples (~150 µL) were collected serially through a jugular vein catheter at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48 h post-dose time points (10 time points) following oral dosing. Samples were collected in K2-EDTA tubes and centrifuged at 4°C for 10 minutes at 13000 rpm to separate the plasma. The plasma samples were immediately transferred to a freezer (-70°C) for storage until bioanalysis. Plasma samples were analyzed using validated LC-MS/MS methods developed in blank matrices as described in section 6.2.4.2.

PK Analysis

The mean plasma concentration versus time graphs were plotted for all three dose groups, and the PK parameters were derived from non-compartmental analysis (NCA) using Phoenix WinNonlin 6.4 version (CERTARA, USA). The parameters included:

a) Maximum observed concentration in plasma (Cmax)
b) Time at which Cmax is observed (tmax)
c) Half-life (t1/2)
d) Mean residence time of the drug (MRT)
e) Area under the curve (AUC) to the last non-zero concentration according to the linear trapezoidal rule (AUC(0-t), where 't' is the corresponding time)
f) AUC(0-∞) (where AUC(0-∞) = AUC(0-t) + AUC(t-∞))
g) AUC_%Extrap_obs

Results and Discussion

Selection of Best Performing Formulation

The dissolution profiles of SN1C and S-SN1C-A200 were compared with the formulation SD2, which is the best amorphous EFZ solid dispersion obtained using EEPO as a carrier. The dissolution profiles are shown in Figure 1.

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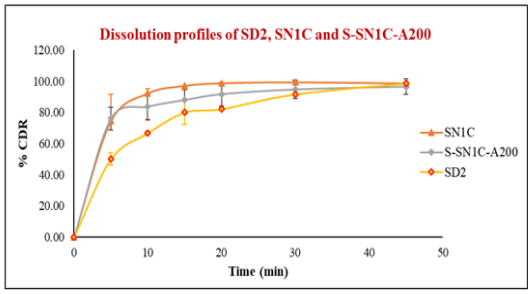

Figure 1. In vitro drug dissolution profiles of SD2, SN1C, and SN1C-A200

The dissolution profile in Figure 1 indicates that the formulation SN1C exhibited the highest dissolution rate. Consequently, SN1C was selected for further investigation in in vivo pharmacokinetic (PK) studies in rats.

Dissolution Profile Comparison with Marketed and Pure EFZ

The dissolution profile of SN1C was compared with both the marketed formulation (MKT) and pure EFZ. The figure clearly demonstrates that SN1C had a significantly higher dissolution rate compared to MKT and EFZ.

Preparation of Calibration Curve

The sample chromatograms of the standard solutions of EFZ and EMP are depicted in Figure 2 and Figure 3. EFZ and EMP were eluted at 1.95 min and 1.76 min, respectively.

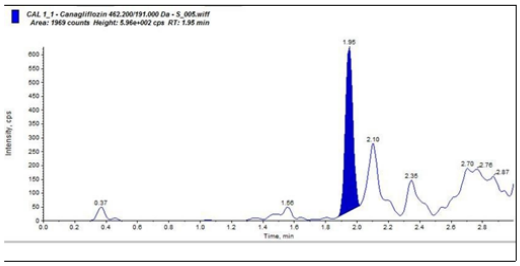

Figure 2. LC-MS/MS chromatogram of the standard solution of EFZ

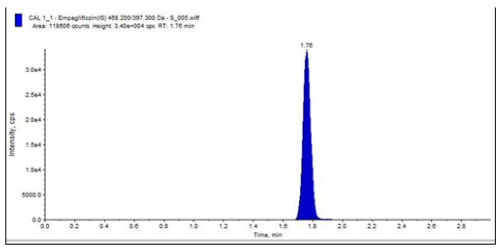

Figure 3. LC-MS/MS chromatogram of the standard solution of EMP (Internal Standard)

The data obtained for plotting the calibration curve for the estimation of EFZ by LC-MS/MS using EMP as the internal standard (IS) is presented in Table 6.

Table 6. Peak area of EFZ using EMP as IS at each analyte concentration

Sr No.	Sample Name	Sample Type	Analyte Concentration (ng/mL)	Analyte Peak Area (counts)	IS Peak Area (counts)	Calculated Concentration (ng/mL)
1	MP	Mobile phase	N/A	0	0	N/A
2	BLK +IS	Double Blank	0	0	582,562	N/A
3	CAL 1_1	Standard	5	2,562	752,632	4.264
4	CAL 1_2	Standard	10	2,563	852,362	7.834
5	CAL 1_3	Standard	50	10,000	745,632	63.17
6	CAL 1_4	Standard	100	18,540	452,632	87.351
7	CAL 1_5	Standard	250	45,847	745,865	123.207
8	CAL 1_6	Standard	500	174,277	745,632	518.368
9	CAL 1_7	Standard	1000	188,747	856,321	1037.776
10	CAL 1_8	Standard	1500	243,789	856,325	1443.92
11	CAL 1_9	Standard	2000	458,023	742,563	1969.029
12	MP	Mobile phase	N/A	0	0	N/A
13	MP	Mobile phase	N/A	0	0	N/A
14	LQC	Quality Control	50	15,245	856,323	50.666
15	MQC	Quality Control	1000	156,223	563,232	1045.233

The linearity curve, plotted using the data in Table 6, is depicted in Figure 4. The regression coefficient was calculated to be 0.9978, which signifies that the method is suitable for the estimation of EFZ in rat plasma.

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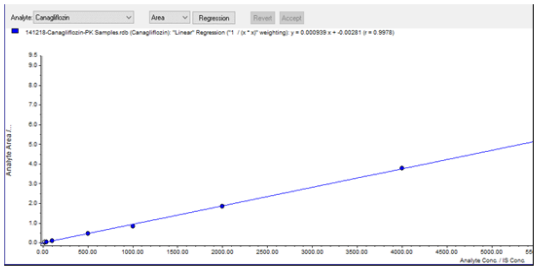

Figure 4. Linearity curve obtained for LC-MS/MS estimation of EFZ

In vivo PK Studies in Rats

The mean plasma concentration versus time data for all three dose groups is presented in Table 7. The corresponding mean plasma concentration versus time graph is shown in Figure 5.

Table 7. Oral PK Data of Empagliflozin for EFZ, MKT, and SN1C

Time (h)	Group 1 (EFZ)	Group 2 (MKT)	Group 3 (SN1C)
	Mean ± STD (ng/mL)	Mean ± STD (ng/mL)	Mean ± STD (ng/mL)
0.25	891.7 ± 92.9	611.6 ± 38.5	303.8 ± 84.6
0.50	1595.4 ± 161.8	1208.4 ± 310.7	642.9 ± 115.0
1.00	2035.8 ± 72.1	1433.1 ± 344.2	1048.6 ± 286.3
2.00	2078.0 ± 321.9	1810.6 ± 339.4	2278.6 ± 288.5
4.00	2426.5 ± 865.3	2438.5 ± 131.3	3990.9 ± 437.1
6.00	1957.5 ± 385.8	2016.7 ± 276.5	2992.4 ± 400.0
8.00	2321.1 ± 238.4	2205.3 ± 78.8	3517.8 ± 659.9
12.00	1596.8 ± 358.5	1525.6 ± 55.0	2362.4 ± 545.1
24.00	414.6 ± 59.4	314.9 ± 79.9	398.3 ± 30.4
48.00	45.7 ± 15.0	35.2 ± 10.8	43.6 ± 17.3

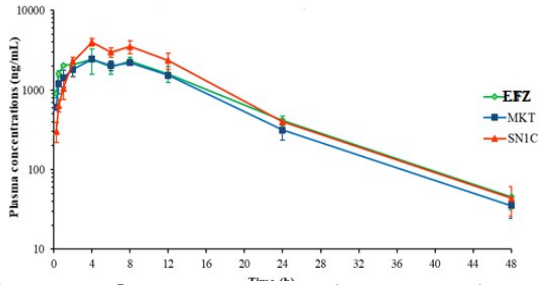

Figure 5. Plasma concentration versus time profile of EFZ, MKT, and SN1C

The mean PK parameters for the three dose groups were derived from non-compartmental analysis (NCA) using Phoenix® WinNonlin® 6.4 (CERTARA, USA). The summary of the PK study results of Empagliflozin in rats, after a single-dose oral administration of 10 mg/kg, is shown in Table 8. The results are expressed as mean ± STD, where n = 3 animals/group.

Table 8. Summary of PK Parameters for Empagliflozin in Rats

PK Parameters	Group 1 (EFZ)	Group 2 (MKT)	Group 3 (SN1C)
Cmax (ng/mL)	3750.7 ± 250.4	3438.5 ± 131.3	5256.3 ± 257.5
tmax (h)	3.23 ± 2.51	3.0 ± 0.0	5.33 ± 2.31
AUC(inf) (h*ng/mL)	49212 ± 7085.6	45441.4 ± 3439.1	62563.1 ± 7311.1
AUClast (h*ng/mL)	48523.5 ± 6949	45096.2 ± 3341	60236.5 ± 7133.8
AUC_%Extrap (obs)	2.2 ± 0.3	2.0 ± 0.3	1.8 ± 0.3
t1/2 (h)	6.0 ± 0.4	6.7 ± 0.6	6.4 ± 0.4

The results in Table 8 suggest that the SNEDDS formulation SN1C showed approximately 1.5-fold higher Cmax compared with pure EFZ powder and MKT. The mean values of Cmax obtained for SN1C were significantly greater than those for EFZ and MKT ($p < 0.05$), indicating that EFZ formulated as SNEDDS resulted in a significant increase in absorption compared with EFZ powder and MKT. This could be attributed to the rapid dispersion of EFZ-loaded SNEDDS in aqueous gastrointestinal (GI) fluid, resulting in extremely small lipid droplet sizes that can easily permeate across the GI tract epithelium [1,2].

Additionally, the incorporation of Kolliphor® RH 40, a non-ionic surfactant in SNEDDS, might have increased intestinal permeability by partitioning into the cell membrane and disrupting the structural organization of the lipid bilayer [5,6], leading to enhanced permeation and absorption.

The mean AUC(inf) for the SN1C formulation was significantly higher than that for both EFZ and MKT ($p < 0.05$). AUC(inf) reflects the actual body exposure to the drug following a specific dose. Given that all three formulations were administered at the same dose (10 mg/kg), these results indicate that EFZ formulated as SNEDDS enhanced the extent of drug absorption. The relative bioavailability values for SN1C compared to EFZ and MKT were 1.3 and 1.4, respectively. The mean tmax values for EFZ, MKT, and SN1C were 4.33 ± 3.51 h, 4.0 ± 0.0 h, and 5.33 ± 2.31 h, respectively. These values are consistent with the literature (~4 to 7 h in rats) (European Medicines Agency 2013) and showed no significant difference among the groups ($p > 0.05$), suggesting that the SNEDDS formulation did not significantly affect the rate of absorption. The mean t1/2 values for EFZ, MKT, and SN1C were

7.0 ± 0.4 h, 6.7 ± 0.6 h, and 6.4 ± 0.4 h, respectively, which align with the literature (~6 to 8 h in rats) (European Medicines Agency 2013) and were not significantly different ($p > 0.05$), indicating that the SNEDDS formulation did not impact the elimination kinetics of EFZ [7,8].

Conclusion

The study demonstrated that the SNEDDS formulation SN1C significantly outperforms both the pure EFZ powder and the marketed formulation (MKT) in terms of dissolution rate and absorption. SN1C exhibited a notably higher dissolution rate and enhanced pharmacokinetic parameters, including a significantly greater Cmax and AUC(inf), compared to EFZ and MKT. This improvement can be attributed to the efficient dispersion of EFZ-loaded SNEDDS in the gastrointestinal tract, which facilitates better absorption due to the small lipid droplet size and the presence of Kolliphor® RH 40.

The pharmacokinetic studies in rats revealed that SN1C resulted in approximately 1.5-fold higher Cmax compared to both pure EFZ and MKT, indicating superior absorption. Despite this enhanced absorption, the rate of absorption (tmax) and elimination kinetics (t1/2) were not significantly altered, suggesting that the SNEDDS formulation did not affect these parameters adversely.

Overall, the results support the use of SN1C as a more effective delivery system for EFZ, enhancing its bioavailability and potentially improving therapeutic outcomes. The successful development and evaluation of SN1C highlight the benefits of using SNEDDS technology in optimizing the delivery and efficacy of poorly soluble drugs.

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