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Research article

Design expert software assisted development and evaluation of empagliflozin and sitagliptin combination tablet with improved in-vivo anti-diabetic activities

Md Saddam Hossain [a], Sadia Jahan [a,*], Sad Al Rezwan Rahman [b], Mashiur Rahman [b], Diponkor Kumar [c], Susmita Paul [d], Joy Chandra Rajbangshi [a]

[a] Department of Pharmacy, Faculty of Science, Comilla University, Bangladesh
[b] Bangladesh Reference Institute for Chemical Measurements, Dhaka, Bangladesh
[c] Department of Pharmacy, Faculty of Life and Earth Sciences, Jagannath University, Bangladesh
[d] Pharmacy Discipline, Khulna University, Khulna, Bangladesh

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ABSTRACT

Background: The combination of empagliflozin and sitagliptin to treat type-2 diabetes might be more economical and patient compliance with an additive improvement in glycemic control due to complementary modes of action.

Aim of the study: To design, formulate and optimize an immediate tablet dosage form containing empagliflozin and sitagliptin utilizing statistically reliable study design followed by in-vitro and in-vivo testing.

Method: ology: To determine the effects of copovidone (X1) and croscarmellose sodium (X2) amounts on the dependent variables of disintegration time and percent drug release, the formulation was developed using Design Expert Software v.13's direct compression method-based central composite design optimization study. The formulations' assay, dissolution, friability, hardness, weight variation, disintegration, and anti-diabetic effects were evaluated in comparison to the standard drug. The analysis included the use of high performance liquid chromatography (HPLC) assay methods. Mice were employed to investigate the efficacy of an anti-diabetic drug after they were administered a high-fat diet and two injections of streptozotocin at a dosage of 30 mg/kg BW each.

Results: Formulation of F3 out of nine had all in-vitro parameters at the most satisfactory condition. It was found that assay of the best formulation is 100.99% and 100.19% for empagliflozin and sitagliptin respectively. The disintegration time of F3 was found at 5.32 min. Percentage release of empagliflozin in 30 min was found 89.05% while sitagliptin was with 93.76%. The results showed that administration of F3 significantly reduced FBG (68.61%, $p < 0.0001$), total cholesterol levels (70.29 ± 0.48; $p < 0.0001$), triglycerides (70.20 ± 0.40, $p < 0.0001$); HDL levels (52.50 ± 0.31; $p < 0.0001$), LDL levels (33.34 ± 0.28; $p < 0.0001$), compared to diabetic control, this effect was comparable to metformin treatment.

Conclusion: The direct compression approach has been used to develop, and optimize a new combination tablet incorporating empagliflozin and sitagliptin with better dissolution rate and anti-diabetic action.

* Corresponding author. Department of Pharmacy, Faculty of Science, Comilla University, Cumilla, 3506, Bangladesh.
E-mail address: sadiajahan@cou.ac.bd (S. Jahan).

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1. Introduction

Type 2 diabetes mellitus (T2DM) is distinguished by relative deficit of insulin triggered by defective pancreatic -cell and resistance of insulin in target organs, necessitating intensive management of weight, glucose and lipid concentrations, as well as blood pressure, in order to lessen the risk of comorbidities and disease severity [1]. Since a single glucose-lowering agent can only treat a limited number of pathophysiological targets and does not control blood sugar levels well enough in many cases, most people with T2DM will eventually need to take more than one glucose-lowering drug. This is convenient, easy to take, and reduces the number of drugs they have to take [2]. The 2013 AACE Comprehensive Diabetes Management Algorithm and the 2015 Position Statement of the ADA/EASD both recommend using combination hypoglycemic drugs that work in complementary modes of action [3,4]. Based on prior study findings, combining a sodium glucose co-transporter-2 (SGLT2) and dipeptidyl peptidase-4 (DPP-4) inhibitor, which have complementary glucose-lowering effects, are regarded as a potential therapy option for individuals with poor glycemic control [5].

Empagliflozin is a potent and specific SGLT-2 inhibitor with a favorable safety profile in people with T2DM. It helps regulate steady-state glycemic control by facilitating glucose reuptake from the proximal tubules of the kidney [6]. Empagliflozin is related with a significant decrease in mortality in T2DM patients with a history of cardiovascular illness, as well as a significant reduction in the risk of hospitalization for heart failure. Sitagliptin is a DPP-4 inhibitor that is often administered to people with type 2 diabetes, has an excellent safety profile, with no increased risk of low blood sugar, weight gain, or heart problems [7]. Therefore, therapeutic approaches can aim both sitagliptin, which secretes insulin in a glucose-dependent manner and suppresses glucagon release, and empagliflozin, which lowers glucose level by reducing renal glucose reabsorption and inducing urinary glucose excretion. Even some anti-diabetic agents like sulphonylureas and thiazolidinediones may lead to several adverse effects in combination, including weight gain and hypoglycemia, but the mechanistic pathway of empagliflozin and sitagliptin implies that they can be administered together in combination without any detrimental side effects [8]. Moreover, since both of these drugs have lower rates of bilirubin excretion, combining them is a beneficial approach for the kidney [9,10].

Because of the easy handling, patient compliance, cost-effectiveness, and ease of large-scale manufacturing of oral dosage forms, oral medication is the most advantageous route of drug administration [11]. For simple, time-saving, and cost-effective tableting procedures, the direct compression method is widely used in the pharmaceutical industry [12]. Disintegration time and dissolution rate are two of the most important parameters of immediate-release tablets because they determine how quickly drugs can reach the body's surface area for better bioavailability and therapeutic effectiveness [13]. Selection of excipients is one of the crucial steps in the design of the delivery system to ensure the quality and efficacy of the drug [14]. In terms of manufacturing procedures, excipients may be added into the formulation to aid in drug dissolution [15]. Adding copovidone to formulations can improve hardness, lower ejection force, and reduce friability without jeopardizing dissolution results [16]. Microcrystalline cellulose (MCC) is frequently cited as one of the excellent excipients for enhancing tablet dissolution rates and compressibility by direct compression [17]. Croscarmellose sodium (Acdisol) is a superdisintegrant, used to aid in fast disintegration, which speeds up the dissolution of directly compressed tablets [18].

Scientists use systematic and powerful mathematical tools for formulation development and optimization of a wide range of pharmaceutical dosage forms in order to generate statistically significant interpretation with a fewer number of experiments for design space estimation. The central composite design is an efficient mathematical model that uses response surface methodology (RSM) to assess the interaction between dependent and independent factors in order to develop optimum formulations [19].

The objective of our study was to develop and optimize immediate-release empagliflozin plus sitagliptin (10 mg + 25 mg) tablet formulations by direct compression utilizing central composite design. The effects of the formulation variables at different levels were determined on responses, including drug release. After assessing the pre and post-compression quality attributes of all formulations, the clinical effectiveness of the developed formulation was evaluated throughout the in-vivo approach. There are currently a number of commercially available formulations with combination forms of different anti-diabetic drugs, such as Synjardy (empagliflozin + metformin) and Glyxambi (empagliflozin + linagliptin), but there is no available formulation that combines empagliflozin and sitagliptin. Although various articles have been published that demonstrate the clinical effectiveness of combining sitagliptin with empagliflozin [20–22] this research study is the first to develop and evaluate these two drugs as a final formulation.

2. Materials and methods

The experimental protocol was in compliance with established animal welfare guidelines and approved by the Animal Ethics Committee of Khulna University (Ref. No.- KUAEC-2022/04/06).

Chemicals:

Empagliflozin, Sitagliptin, Metformin was being provided as a gift sample by Beximco Pharmaceuticals Ltd., Bangladesh. Magnesium stearate, copovidone, croscarmellose sodium and microcrystalline cellulose were procured from Spectrum Chemical Mfg. Corp., USA. HPLC grade water and acetonitrile were brought from PT. SMART LAB Indonesia and Streptozotocin (STZ) was procured from Sisco Research Laboratories Pvt. Ltd., India.

Compatibility Study by FT-IR:

Empagliflozin and sitagliptin, as well as a physical blend of APIs and excipients, were combined with IR grade KBr pellets. The sandwiched plates were being put in an FT-IR spectrometer (PerkinElmer LS55, USA) to collect spectra between the wavelengths of 4000–400 cm^{-1} [23].

Pre-Compression Studies of Powder Blends:

The initial phase in drug development is pre-formulation research was carried out to reduce inaccuracies and provide data for the

development of dosage forms. To calculate the angle of repose, the fixed funnel approach was utilized. The formula, which determines the equation for computing the bulk density, is the ratio of the bulk mass of powder to the bulk volume. Using tapped density equipment, the measurement cylinder containing the porous mass of powder was tapped. The % compressibility of the powder combination was calculated using the apparent bulk density and the tapped density [24].

Preparation of model formulations:

To conveniently optimize the formulation and evaluate the effect of excipients on hardness and disintegration time, the central composite design [25] was used to prepare structured model formulations consisting of two process parameters: copovidone (X1) content and croscarmellose sodium content (X2). The active pharmaceutical ingredients (empagliflozin and sitagliptin) and other formulation components (microcrystalline cellulose and magnesium stearate) remained constant. The total amount of changing and constant components remained constant at 300 mg. Design-Expert software version 13.0 placed 9 model formulations randomly based on the center composite model. Table 2 summarizes the components of all model formulations. To achieve total drug release, the percentage disintegration time and dissolution rate were chosen as response variables. Direct compression was used to generate all of the tablets containing 10 mg of empagliflozin and 25 mg of sitagliptin. In a mortar, accurately weighed quantities of medications and excipients were geometrically combined. This mixture was well stirred in a polythene bag for 15 min after being put through a no. 40 sieve. After 2 min of lubrication with magnesium stearate, the powder blend was crushed into tablets using a 9-station rotary tableting machine utilizing 9-mm round, flat-faced punches. The matrix tablets had a total weight of 300 mg (Table 2) with varying drug-excipient ratios.

2.1. Post-compressional studies of prepared matrix tablets

The appearance, thickness, weight fluctuation, hardness, and friability of the matrix tablets were all examined. The thickness of the tablet was measured by placing it vertically between two jaws of Vernier calipers. It is measured in millimeters. Individual tablet weights were compared to the average weight of twenty tablets to assess weight variance. The Monsanto hardness tester was used to measure the hardness of six tablets. It is expressed in kilograms per square centimeter. To examine the friability criterion, compressed tablets that lost less than 0.5–1.0% of their weight following rotation were typically deemed acceptable. It is represented as a percentage (%) and is computed using the following formula: Friability (%) = Initial weight − Final weight/Initial weight 100 [26].

For the tablet disintegration test, one tablet was inserted in each of the six tubes of the disintegration test apparatus (Veego, India), and the basket rack was placed in a 1 L beaker of 37 °C water. It was timed how long it took for all particles to pass through the 10 nm mesh screen [27].

2.2. Determination of content (assay) of empagliflozin and sitagliptin with high-performance liquid chromatography (HPLC)

To determine the assay of empagliflozin and sitagliptin in tablet formulation same HPLC chromatographic method was followed. The content of empagliflozin and sitagliptin, which have antidiabetic activity, was tested for each formulation using an HPLC isocratic flow (1 ml/min) system, LC-2030C 3D Plus pump (Shimadzu, Japan), column C18 (250 mm x 4,6 mm, 5 μm, Shimadzu, Japan), and a UV detector (228-65802-48). Furthermore, the HPLC method was performed with reference to Refs. [28–30] with modification. The optimal wavelength was set at 254 nm. The mobile was consisted of phosphate buffer with pH 6.4 and acetonitrile (60:40 ratio) with 20 μL injection volume maintaining at 30 °C temperature. The mobile was used as the blank solution.

2.3. % release of drugs (dissolution) study

The dissolution test was done on all formulations with 900 ml of 0.1 N hydrochloric acid using USP type-II equipment (Veego, India). At 100 rpm and 37 °C±0.5 °C, the solution has been used as a dissolution medium. The samples were evaluated using the UV–Visible spectroscopic technique (Shimadzu Corporation, Japan) at 247 nm for empagliflozin and 267 nm for sitagliptin, respectively, and the percentage dug release was determined [31,32].

2.4. Selection of animal

The experiment was carried out on thirty adult Swiss albino mice weighing 25–30 g on average and kept in colony cages at an ambient temperature of 25 ± 2 °C with a 12-h-light/12-h-dark cycle. The mice were obtained from the Animal Research Branch of the International Centre for Diarrheal Disease and Research in Bangladesh (ICDDRB). The mice were fed a regular pellet meal (Amrut rat and mice feed, Sangli, India) and had unlimited access to water. Throughout the trial, the Principles of Laboratory Animal Care (Health, 1985) were followed. Normoglycemic mice were chosen for this experiment because their fasting blood glucose level was less than 150 mg/dl [33].

2.5. Induction of experimental diabetes

After one week of acclimation, five mice were fed a conventional pellet diet, while the others were fed a High Fat Diet (HFD: 20.07% fat, 17.78% protein, and 2.75% crude fiber). The rats (N = 20) were starved overnight after three weeks of HFD treatment before being induced with diabetes through an intraperitoneal injection of STZ at a twofold dose of 30 mg/kg BW (1 ml/kg) at one-week intervals. Citrate buffer was given to normal control animals. Furthermore, each time STZ was injected, it was newly produced in

cold citrate buffer (pH 4.5, 0.1 M). Ten days following the second STZ injection, their fasting blood glucose (FBG) level was tested. Blood was obtained from the tip of the mouse's tail and smeared on a test strip. The fasting blood glucose (FBG) level was measured using a glucometer (Accu-Check Active, Roche Diagnostics, Mannheim, Germany). HFD was administered to the induction mice group until the end of the study. The formula [34] for calculating the blood glucose level decrease was:

(%) = 100 × (Cn–Ct)/Cn, where Cn = Average blood glucose level in diabetic control group, and Ct = Average blood glucose level treated with metformin, placebo and test formulation.

2.6. Antidiabetic activity test

Twenty-five mice were divided into 5 groups, as follows: 1. Normal control (NC), non-diabetic mice administered with vehicle solution 2. Diabetic control (DC), diabetic mice administered with vehicle solution 3. Positive control, diabetic mice treated with metformin 250 mg/kg BW. 4. diabetic mice treated with placebo (test formulation without active pharmaceutical ingredients). 5. diabetic mice treated with the test formulation (empagliflozin sitagliptin; 25 mg + 10 mg). To select the sample size for conducting in-vivo study previous investigations have been followed base on the exclusion or inclusion criteria of animal models [35]. All treatments were administered orally once daily, for fifteen consecutive days. The FBG levels were measured on days 1,5,10 and 15 after treatment. Serum lipid profiles (cholesterol, triglyceride, HDL, and LDL) were determined using test kits obtained from Merck, India.

3. Result and discussion

3.1. Compatibility Study by FT-IR

Compatibility studies of pure drugs empagliflozin and sitagliptin with excipients were carried out prior to the preparation of tablets through FTIR analysis. Since, several previous studies have clearly reported the compatibility between empagliflozin and sitagliptin with our [36–38] chosen excipients, here we only did the investigations to determine that if the drug-excipients are compatible in as a formulation. In all, three samples were injected into the instrument: two of them had two APIs separately, while the third included all of the excipients together with the APIs. All the characteristic peaks (Figs. 1–3) of empagliflozin and sitagliptin were present in the spectra of the formulation, blend thus indicating compatibility between the drugs. It shows that there was no significant change in the chemical integrity of the drug.

3.2. Formulation development studies

During formulation development, there are many suitable approaches to selecting the excipients that are the most compatible with active pharmaceutical ingredients, for example MCC-PH 102 was selected because of its better mechanical properties during direct compression process [39]. Another approach used for excipient selection was that the most commonly used excipients in different empagliflozin and sitagliptin formulations, as shown in Table 1, in order to improve the dissolution rate of the final formulation. Design Expert Software (version 13.0) that is specifically dedicated to performing design of experiments (DOE), recommended the percentage composition of each ingredient. The most widely used central composite design has been adopted because maximum information can be obtained with minimum trial by this method [25]. Different formulation trials were randomized and presented in Table 2. The powder blend was evaluated for various parameters as shown in Table 3. This showed that powder blends from all the formulations had good flow properties based on standard values [40]. It indicates that direct compression can be employed because of the good flow ability of the powdered mixture [41]. Magnesium stearate was incorporated to ensure uniform flowability and easy ejection of units after compression.

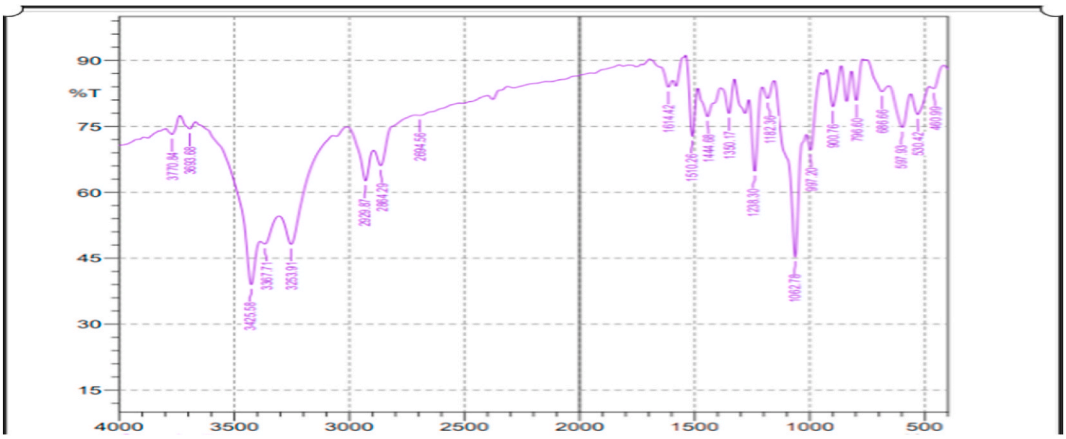

Fig. 1. FT-IR chromatogram of empagliflozin powder.

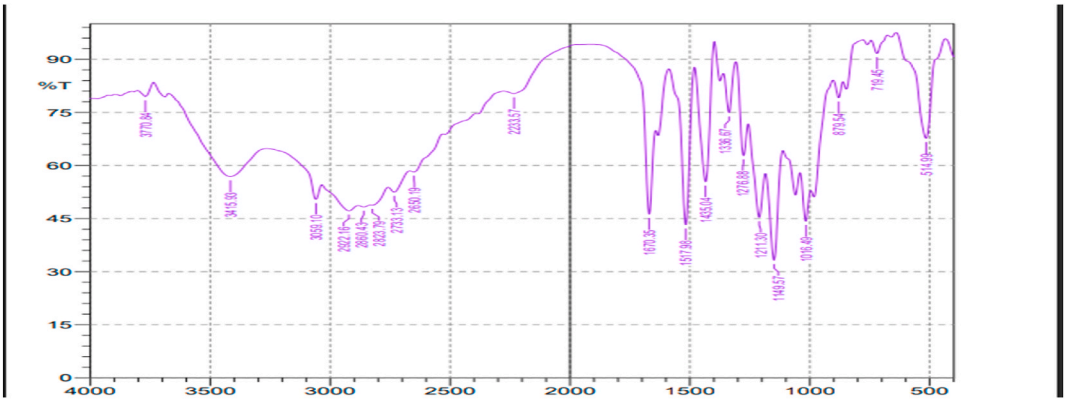

Fig. 2. FT-IR chromatogram of sitagliptin powder.

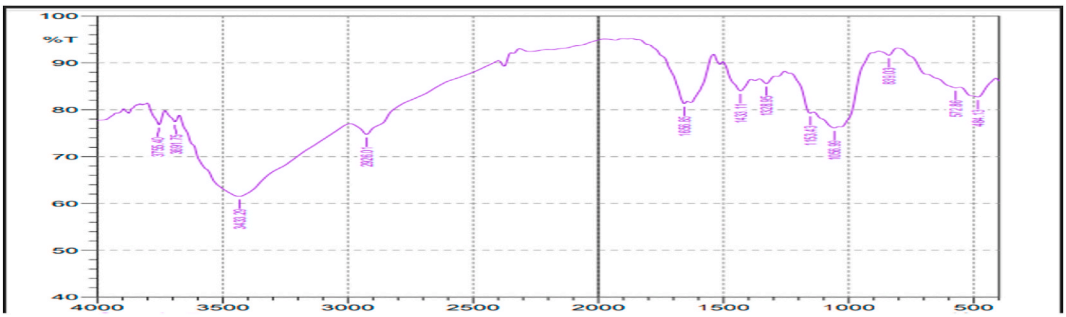

Fig. 3. FT-IR chromatogram of powder blend.

Table 1
The proposed formulation included the following ingredients.

Ingredient	Function
Empagliflozin	Active Ingredient
Sitagliptin	Active Ingredient
Copovidone	Binder
Croscarmellose Sodium	Disintegrant
Magnesium Stearate	Lubricant
Microcrystalline Cellulose (Avicel PH 102)	Filler

Table 2
Amount (in mg) compositions of excipients in different formulation trials.

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
Empagliflozin	10	10	10	10	10	10	10	10	10
Sitagliptin	25	25	25	25	25	25	25	25	25
Copovidone	11	13	12	11	10.5	13.5	12	12	13
Croscarmellose Sodium	23	23	25.5	25	24	24	24	22.5	25
Magnesium Stearate	6	6	6	6	6	6	6	6	6
Microcrystalline Cellulose (Avicel PH 102)	225	223	221.5	223	224.5	221.5	223	224.5	221

- Angle of repose is: tan-1 (2 h/d)
- Bulk density = Weight of powder bulk/Bulk volume
- Tapped density = Weight of powder blend/Tapped volume of packing
- Carr's index = Tapped density - Bulk density × 100/Tapped Density
- Hausner's Ratio: Hausner's ratio = Tapped density/Bulk density [42,43]

All the formulations showed satisfactory results as shown in Table 4(a) with respect to hardness, friability, weight variation, assay

Table 3
Pre-compression parameters of powder blend.

Formulation	Angle of Repose (°)	Bulk Density (g/cm^3) (Mean ± SD)	Tapped Density (g/cm^3) (Mean ± SD)	Compressibility Index (%)	Hausner's Ratio
F1	23.72	0.45 ± .01	0.51 ± 0.01	13.33	1.33
F2	24.28	0.45 ± .01	0.51 ± 0.01	13.33	1.33
F3	23.51	0.44 ± 0.02	0.51 ± 0.02	15.91	1.15
F4	22.35	0.46 ± 0.01	0.52 ± 0.01	13.04	1.13
F5	24.5	0.44 ± 0.02	0.51 ± 0.02	15.91	1.16
F6	24.11	0.45 ± 0.02	0.51 ± 0.02	13.33	1.13
F7	21.92	0.47 ± 0.02	0.53 ± 0.02	12.76	1.13
F8	24.09	0.45 ± 0.02	0.51 ± 0.02	13.33	1.13
F9	24.14	0.44 ± 0.02	0.50 ± 0.02	13.63	1.14

and in vitro disintegration time except for the percentage release. Only two formulations (F3, and F7) released more than eighty-five percent (85%) of empagliflozin. For sitagliptin also, F3 showed highest %release at 30 min. The dissolution profile of the developed formulation ensures the intrinsic ability of the drug to dissolve in the fluids of the gastrointestinal tract which is a crucial parameter for drug development [44] (see Table 4(b)).

Results from high performance liquid chromatographic (HPLC) analysis have demonstrated that the retention times of empagliflozin and sitagliptin in the samples were similar to the reference standard. There was no interference found between the excipients and APIs, as demonstrated in Figs. 4–6. For assay tests through the HPLC method, the average retention time for empagliflozin and sitagliptin was found to be around 5.4 min and 4.1 min, respectively.

The tablet formulation in this study was performed by applying a central composite rotatable design with two independent components (binder and disintegrant). This is one of the effective statistical methods that gives polynomial quadratic interpretation to explore the impacts of formulation factors (independent) on product quality (dependent). Copovidone (binder) and croscarmellose sodium (disintegrant) concentrations are formulation variables denoted as X1 and X2, respectively. By rotating the variables below (−1) and above (+1) the median of two factor levels, nine formulations were developed in total.

Because of the increased binder concentration, the hardness of the testing was determined to be the highest in F6. Copovidone addition increased tensile strength at a given compression pressure [45].

Though microcrystalline cellulose (MCC) can contribute in disintegration, a strong disintegrant is still required in the formulation [46]. Due to the sheer greater dose of croscarmellose sodium, F3 disintegrated faster. Because of the increased binder concentration, formulation Trial F6 decomposed quite slowly. The rapid disintegration of F3 provides a rapid clinical response without any adverse effects like stomach disturbances or nausea caused by parenteral administration of GLP-1 agonist.

The formulation (F3), which releases more than 85% as per USP for both empagliflozin and sitagliptin, was recommended out of all formulations for further investigation.

The model F-value of 9.48 (p value 0.0467) denotes the model is significant for disintegration time. This F-value might be caused by noise only in 4.67% of cases.

The model F-value of 12.06 (p value 0.0336) denotes the model is significant for the empagliflozin dissolution rate (% release in 30 min). This F-value might be caused by noise only in 3.36% of cases.

The model F-value of 12.09 (p value 0.0334) denotes the model is significant for the sitagliptin dissolution rate (% release in 30 min). This F-value might be caused by noise only in 3.34% of cases.

Excipient interaction was examined utilizing response surface methods using central composite design. figures (7-9) revealed the rotation and interference of excipients versus the response variables of disintegration time and dissolution rate. The interactions of excipients and their impacts on reactions are reflected in various lines and shadings. Below, in coded terms, are the quadratic equations (i-iii) for the dependent variables disintegration time (Y1), empagliflozin dissolution rate (Y2), and sitagliptin dissolution rate (Y3);

(i) $Y1 = 5.72 + 0.1963*A+0.0087*B+0.0175*AB+0.6306\ A^2-0.1744\ B^2$
(ii) $Y2 = 88.47 + 1.24*A+1.19*B-0.2550*AB-5.02*A^2-1.13*B^2$
(iii) $Y3 = 93.15 + 1.31*A+1.25*B-2700*AB-5.28*A^2-1.19*B^2$

Table 4 (a)
Post-compression parameters of formulation F1–F9.

Formulation	Tablet hardness (kg/cm2)	Friability (%)	Weight variation (% RSD)	Disintegration time (minutes)
F1	6	0.25	300.2	6.15
F2	6.1	0.33	297.4	6.38
F3	6.37	0.42	300.8	5.32
F4	6.08	0.17	298	6.27
F5	6.16	0.17	299.6	7.33
F6	6.42	0.33	297.4	7.1
F7	6.28	0.25	299.8	6.12
F8	5.8	0.08	300.64	5.49
F9	6.06	0.5	297	6.57

Table 4 (b)
Post-compression parameters of formulation F1–F9.

Formul-ation	% release of empagliflozin in 30 min	% release of sitagliptin in 30 min	Assay (%) of empagliflozin	Assay (%) of sitagliptin
F1	80.23	84.47	100.79	100.01
F2	82.16	86.51	99.85	99.05
F3	89.05	93.76	100.99	100.19
F4	82.11	86.45	100.05	99.26
F5	76.38	80.41	100.59	99.87
F6	81.39	85.69	99.85	99.05
F7	88.47	93.15	100.65	99.86
F8	84.27	88.73	100.94	100.14
F9	83.02	87.41	99.72	98.93

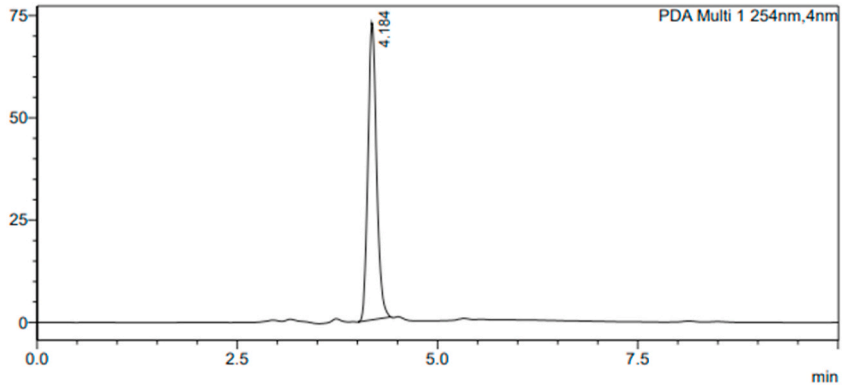

Fig. 4. HPLC chromatogram of sitagliptin.

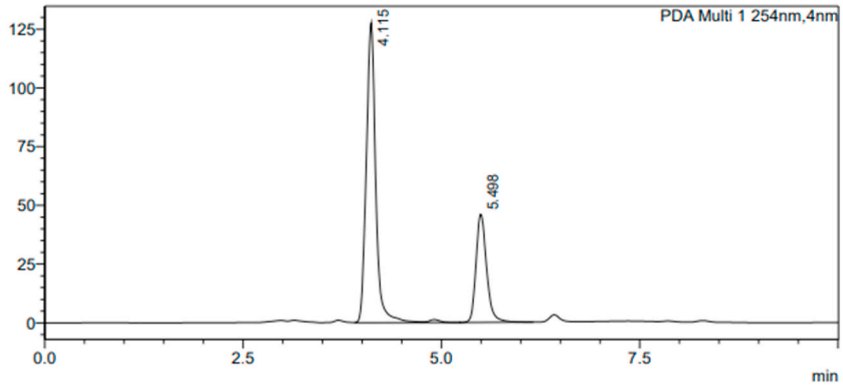

Fig. 5. HPLC chromatogram of empagliflozin.

3.3. Body weight after treatment

The body weight was significantly recovered after 15 days of F3 therapy, although the placebo and standard groups were insignificantly different from the DC group (Table 5). After the test sample was treated, no significant changes in the NC group were identified.

STZ-induced diabetes causes body weight loss due to increased muscle wasting and tissue protein loss [47]. The antidiabetic activity of the test formulation was further elucidated by the findings obtained in a diabetic model using a combination of empagliflozin and sitagliptin therapy. Gain in body weight was found after 15 days of therapy, and the findings were analogous to those of the standard medication glibenclamide.

3.4. Blood glucose after treatment

Before treatment, all animals' fasting blood glucose (FBG) levels were within acceptable limits. In comparison to the NC group, FBG

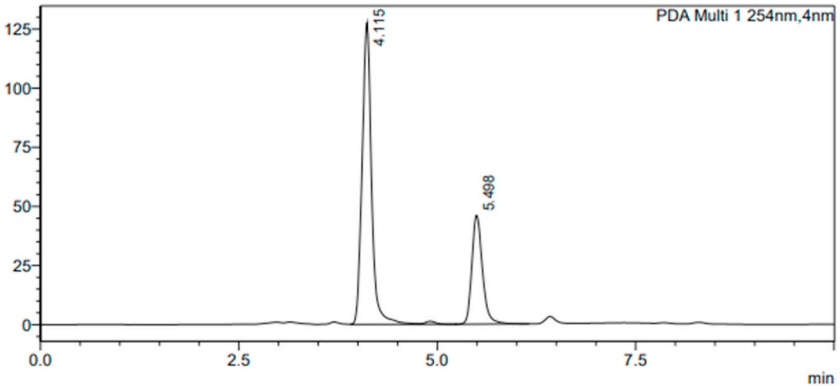

Fig. 6. HPLC chromatogram of empagliflozin and sitagliptin.

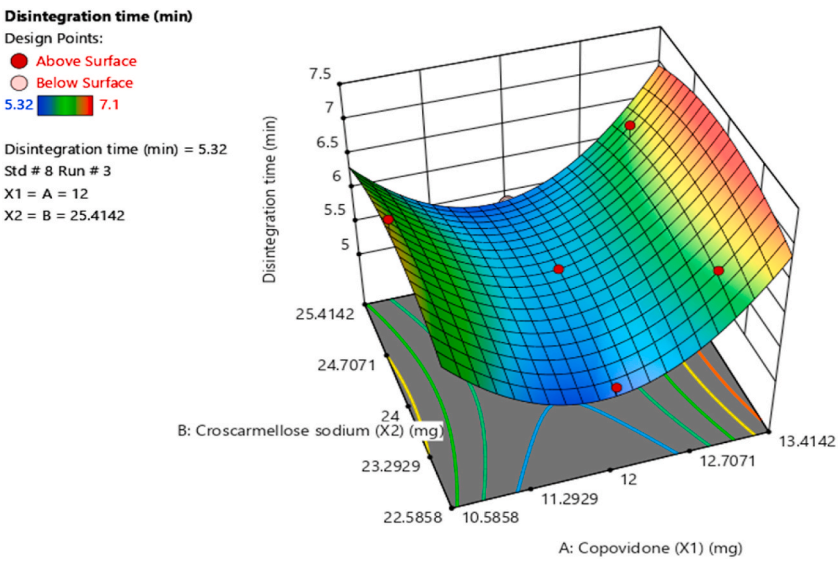

Fig. 7. Response surface plots presenting the effects of copovidone (X1) and croscarmellose sodium amount (X2) on disintegration time.

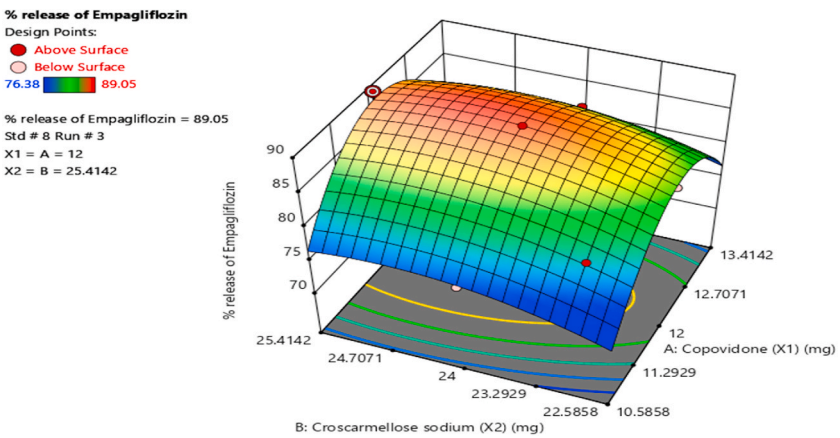

Fig. 8. Response surface plots presenting the effects of copovidone (X1) and croscarmellose sodium amount (X2) on dissolution rate (% release in 30 min) of Empagliflozin.

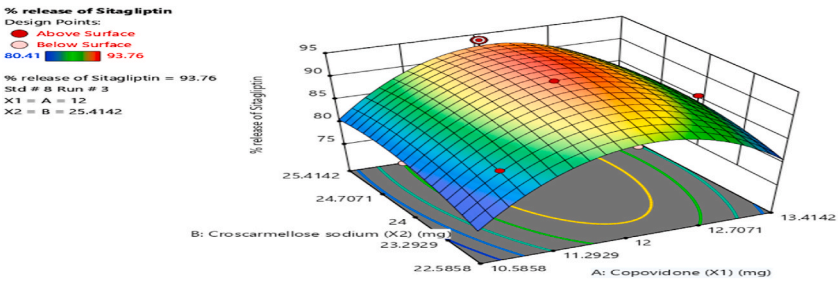

Fig. 9. Response surface plots presenting the effects of copovidone (X1) and croscarmellose sodium amount (X2) on dissolution rate (% release in 30 min) of sitagliptin.

Table 5
Effect of F3 on body weight in STZ-induced diabetic mice.

Group Treatment (n = 5)	Changes in body weight (g)			
	1st day	5th day	10th day	15th day
Normal Control (NC)	31.45 ± 0.38	31.85 ± 0.29	32.38 ± 0.11	33.13 ± 0.09
Diabetic Control (DC)	34.52 ± 0.71	34.18 ± 0.84	34.16 ± 0.78 ^{ns}	32.7 ± 0.67 ^{ns}
Placebo	32.52 ± 0.73	33.28 ± 0.87^{ns}	33.68 ± 0.87^{ns}	33.72 ± 0.96^{ns}
Standard (Metformin)	31.32 ± 0.64	32.7 ± 0.86^{ns}	33.14 ± 0.90^{ns}	32.66 ± 0.93^{ns}
Test formulation (F3)	30.36 ± 0.75	30.06 ± 0.91 ^{ns}	30.08 ± 0.83 ^{ns}	29.44 ± 0.73*

Significantly different from DC group (*p < 0.05). Data are expressed as the mean ± SEM (n = 5), when compared with diabetic control by using one way ANOVA followed by Dunnette's multiple comparison test.

levels were considerably higher after 24 h after STZ injection. FBG levels were significantly different from DC levels after 10 and 15 days of F3 administration, however there was no significant impact for placebo. When compared to the DC group, the standard (metformin) and F3 groups showed a 65.17% and 68.61% (p<0.0001) decline in blood glucose levels at the culmination of 15 days of therapy, respectively (Table 6 and Fig. 10). Apparently, combining empagliflozin and sitagliptin had a significant additional glucose-lowering benefit. Empagliflozin blocks reabsorption through the kidneys, allowing excess glucose to be removed, whereas sitagliptin reduces blood sugar levels by boosting insulin and reducing glucagon [48]. Hence, the developed formulation could be an ideal hypoglycemic agent as it has been reported to decrease blood glucose more effectively than metformin, which is considered first-line therapy to treat type-2 diabetes [49].

3.5. Lipid profile after treatment

When compared to the DC or NC group, the F3 resulted in a significant reduction in total cholesterol, low density lipoprotein (LDL), and triglyceride (TG) levels (Table 7). When compared to the NC and DC groups, F3 therapy resulted in a significant rise in HDL levels (p < 0.0001). But, in the placebo group, there were no significant changes observed compared to the DC group for any parameters. In this study, the induction of STZ among mice changed the lipid profiles significantly for all groups. The lipid profile in this study is consistent with diabetic dyslipidemia, which is defined by high TC, high TG, low HDL cholesterol, and elevated LDL cholesterol levels [50]. After the treatment, the value of the lipid profile was considered within the normal range [51]. Numerous studies suggest that empagliflozin lowers blood cholesterol and even slows the progression of atherosclerosis in people with diabetes [52,53]. In individuals with T2DM, sitagliptin, alone or in combination with the other hypoglycemic drugs, markedly enhances blood TG and HDL levels [54]. Diabetic dyslipidemia management is a well-known preventive strategy for treating type 2 diabetes by lowering cardiovascular risk [55]. But some commonly available marketed anti-diabetic drugs have serious adverse effects on cardiovascular health.

Table 6
Effect of F3 on blood glucose level in STZ-induced diabetic mice.

Group Treatment (n = 5)	Before treatment	Fasting blood glucose (mg/dl) ± SEM			
		1st day	5th day	10th day	15th day
Normal Control (NC)	109.29 ± 0.79	107.85 ± 0.65	109.93 ± 0.83	103.94 ± 0.84	102.5 ± 0.74
Diabetic Control (DC)	105.12 ± 0.42	300.18 ± 0.79	299.48 ± 0.99$^{####}$	300.8 ± 0.83$^{####}$	297.80 ± 0.79$^{####}$
Placebo	103.64 ± 0.77	298.79 ± 0.48	298.60 ± 0.30$^{####ns}$	298.85 ± 0.53$^{####ns}$	297.72 ± 0.47^{ns}
Standard (Metformin)	103.48 ± 0.78	295.64 ± 0.84	222.13 ± 0.95$^{####****}$	148.44 ± 0.68$^{####****}$	103.74 ± 0.38 ****
Test formulation (F3)	104.66 ± 0.94	297.60 ± 0.94	219.84 ± 0.70$^{####****}$	144.96 ± 0.87$^{####****}$	93.48 ± 0.68$^{####****}$

Significantly different from NC group ($^{####}$p < 0.0001) and significantly different from DC group (****p < 0.0001). Data are expressed as the mean ± SEM (n = 5), when compared with diabetic control by using one way ANOVA followed by Dunnette's multiple comparison test.

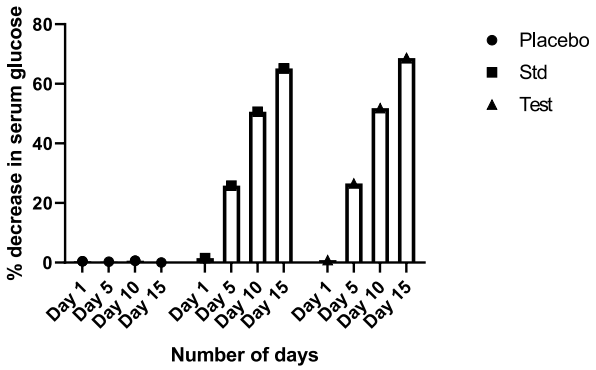

Fig. 10. Effect of F3 on blood glucose level in STZ-induced diabetic mice.

Table 7
Effect of F3 on lipid profile level in STZ-induced diabetic mice.

Treatment Group	Total Cholesterol	Triglyceride (TG)	HDL	LDL
Normal Control (NC)	88.95 ± 0.31	81.15 ± 0.45	69.71 ± 0.24	43.47 ± 0.19
Diabetic Control (DC)	98.7 ± 0.4$^{\#\#\#\#}$	94.6 ± 0.80$^{\#\#\#\#}$	48.46 ± 0.40$^{\#\#\#\#\#}$	51.08 ± 0.66$^{\#\#\#}$
Placebo	98.5 ± 0.2$^{\#\#\#\#}$	92.35 ± 0.45$^{\#\#\#\#}$	48.58 ± 0.33$^{\#\#\#\#\#}$	51.88 ± 0.84$^{\#\#\#}$
Standard (Metformin)	78.25 ± 0.45$^{\#\#\#\#****}$	75.95 ± 0.45$^{\#\#****}$	53.26 ± 0.39$^{\#\#\#\#\#****}$	37.67 ± 0.45$^{\#\#****}$
Test Formulation (F3)	70.29 ± 0.48$^{\#\#\#\#****}$	75.20 ± 0.40$^{\#\#****}$	52.50 ± 0.31$^{\#\#\#\#\#****}$	33.34 ± 0.28$^{\#\#\#****}$

Significantly different from NC group (##p < 0.01; ###p < 0.001; ####p < 0.0001) and significantly different from DC group (****p < 0.0001s). Data are expressed as the mean ± SEM (n = 5), when compared with diabetic control by using one-way ANOVA followed by Dunnette's multiple comparison test.

that lead to even heart failure. For example, one study reported that when compared to controls, rosiglitazone was linked to a 33% higher risk of cardiovascular events, which includes heart attack, heart failure, cardiovascular and non-cardiovascular related mortality. The 274 events among 11,837 rosiglitazone patients and 219 events among 9319 control patients were utilized to estimate this [56]. As a result, the developed formulation combining empagliflozin and sitagliptin can be utilized to treat diabetes without any serious cardiac adverse effects.

4. Conclusion

In terms of in-vitro parameters, the developed combination formulation (F3) containing empagliflozin and sitagliptin met all acceptance criteria. It was observed that the formulation that contained the highest amount of disintegrant had the most satisfactory results. An optimal amount of binder was also added to the formulation to get the best outcome. The formulated tablets' in-vivo hypoglycemic efficacy was also shown to be comparable to the standard product available. The developed formulation was found pharmacologically effective without any adverse effects on body weight or cardiac health. All of the results suggest the developed formulation as a better treatment option for diabetes mellitus as a combination immediate oral dosage form. As a result, an interested local pharmaceutical business in the country may manufacture it as a tablet formulation.

The fact that the entire investigation was performed during the pandemic contributes to the study's limitations. As a result, the formulation contains only one dose of empagliflozin and sitagliptin, both of which are recommended as starting dose [57,58] when delivered in a single dosage form. In this case, an in-depth analysis at several dose levels should be performed to obtain the most effective clinical response.

4.1. Recommendation

If this new formulation is to be commercialized, stability and bioequivalence tests must be conducted. Furthermore, government financial assistance is required to implement the research steps successfully.

Author contribution statement

Md. Saddam Hossain: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sadia Jahan: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sad Al Rezwan Rahman: Analyzed and interpreted the data, Wrote the paper.

Mashiur Rahman, Diponkor Kumar, Susmita Paul, Joy Chandra Rajbangshi: Contributed Reagents, Performed the experiments.

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Data availability statement

The authors are unable or have chosen not to specify which data has been used.

Declaration of interest's statement

The authors declare no conflict of interest.

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