IN VIVO EVALUATION OF OPTIMIZED FORMULATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN BILAYERED TABLETS

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ABSTRACT

Combining dapagliflozin and saxagliptin presents a promising strategy for managing type 2 diabetes mellitus by leveraging their distinct, yet complementary, mechanisms of action. A novel bilayer tablet with a 5 mg sustained release layer of saxagliptin and a 10 mg immediate release layer of dapagliflozin was developed. Live animal studies (*in vivo*) were conducted on rabbits to evaluate the effects of an optimized formulation. Based on *in vivo* performance, the novel bilayer tablets demonstrated greater bioavailability. A new, easy-to-use technique was created to simultaneously measure the two drugs (dapagliflozin and saxagliptin) in rabbit blood plasma. Evaluation of the technique's parameters were done on rabbit plasma in accordance with ICH guidelines. The parameters for pharmacokinetic analysis were ($AUC_{0-\infty}$), (C_{max}), T_{max} and others. Direct calculations of the C_{max} and T_{max} were made using experimental plasma concentration versus time data. The $AUC_{0-\infty}$ was produced by adding the computed AUC_{0-24h} using the trapezoidal rule. Using sample analysis of variance or independent sample t tests, average data variation was compared (one way analysis of variance). Statistical significance (p=0.05) was assessed using a 95% confidence interval.

Keywords: Dapagliflozin, saxagliptin, bilayer tablets, plasma, formulation

INTRODUCTION

Bilayer tablets offer a solution for combining two medications that require different release profiles or cannot be directly mixed due to incompatibility. Each layer releases one medication separately, ensuring optimal delivery and avoiding potential interactions. The addition of a controlled release component is often compacted onto an immediate release granulate after which it has been compressed. The first tablet offers a bilayer action in a typical ultimate dosage form. Drug release in the case of bilayered tablets may be nearly unidirectional[1-4]. A chronic, progressive metabolic condition called type 2 diabetes mellitus (T2DM) is defined by an inadequate supply of insulin[5]. In recent years, there have been a number of novel pharmacological compounds that have been developed to effectively control diabetes by either utilizing individually or in combination. One such combination which was made available in 2017 is dapagliflozin and saxagliptin.

Dapagliflozin, works by blocking a protein called SGLT-2. This protein, found mainly in the kidney's proximal tubules, is responsible for reabsorbing sugar from the urine back into the bloodstream. By blocking SGLT-2, dapagliflozin helps the body to eliminate excess sugar through the urine, lowering blood sugar levels. The kidneys normally reclaim most sugar (glucose) from the urine. Drugs that block this process, like SGLT2 inhibitors, cause some sugar to be passed out instead, leading to better blood sugar control[6,7].

Saxagliptin, works by boosting natural hormones called incretins. These hormones helps to lower blood sugar by directing the pancreas to create more insulin and the liver to make less sugar[8,9].

Dapagliflozin and saxagliptin combination is used together with proper diet and exercise to treat type 2 diabetes[10]. Developing a single-tablet formulation combining these medications could offer a promising solution for enhancing blood glucose management and potentially increase patient adherence to therapy. A unique bilayer pill was created that contained a layer with 10 mg

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of dapagliflozin for immediate release and a layer with 5 mg of saxagliptin for delayed release[11].

MATERIALS AND METHODS

The regulatory authority for Control of Experiments on Animals authorized the *in vivo* study methods for the optimised formulation. The report from the Institutional Animal Ethics Committee previous approval (Ref: P7/IAEC/2016/II/VVIPS/VR) was obtained to carry out the studies. The optimized bilayer tablet, which consists of an immediate release layer containing 10 mg of dapagliflozin and a sustained release layer containing 5 mg of saxagliptin, was chosen as the dosage form for administration. These tablets were created in a lab setting under strict control, and they were chosen based on stability and *in vitro* release experiments.

Preparation of immediate release layer

The preparation of the immediate release layer, which contains dapagliflozin, was prepared by weighing super disintegrants sodium starch glycolate, Laycoat® RS 720, Ludiflash®, PVP K30 and MCC, sifting them through sieve No. #40 (ASTM), blending them in a poly bag for 10 minutes and lubricating them with talc and magnesium stearate. They were then sieved #60 (ASTM), and mixed them in the same poly bag for 2-3 mins.

Preparation of sustained release layer

By weighing saxagliptin, SR polymer (HPMC K15M, Carbopol 940 and gum karaya), PVP K30, MCC and sifting them through sieve no. 40 (ASTM), as well as lubricating them with talc and magnesium stearate that were passed through sieve no. 60 (ASTM) and blending them in the same poly bag for an additional two to three minutes, the lower SR layer for sustained release was created. The composition of bilayered tablet formulation is shown in Table I.

Validation of the bioanalytical method

Method validation was done in rabbit plasma in accordance with ICH guidelines[12-13].

Selectivity

To ensure that there were no conflicting peaks at the R_t of dapagliflozin, saxagliptin, and IS, selectivity was tested using blank plasma samples.

Calibration, linearity, and quality control samples

Using standards that included eight different concentrations, calibration curves were obtained every day for three days. The peak-area ratios of dapagliflozin and saxagliptin to IS were calculated to create curves. Working solutions for the creation of calibration standards were prepared in centrifuging tubes, combining 0.5 mL of blank plasma with 0.5 mL of standard stock solutions of dapagliflozin and saxagliptin at the concentrations of 50, 100, 300, 1250, 2500, 3000, 4000, and 5000 ng mL^{-1} of dapagliflozin. To do this, 1 mL of acetonitrile was mixed with 0.5 mL of internal standard in a cyclomixer for 15 seconds. It was vortexed for 2 minutes, and finally centrifuged at 3200 rpm for 5 minutes. To generate 10, 20, 30, 250, 500, 600, 800, and 1000 ng mL^{-1} of dapagliflozin and 5, 10, 15, 125, 250, 400, 800, and 1000 ng mL^{-1} of saxagliptin concentration, the organic layer was extracted after centrifugation.

Table I: Composition of optimized dapagliflozin and saxagliptin bilayered tablets

Composition of dapagliflozin (IR) layer and saxagliptin (SR) layer of bilayered tablets			
Ingredient	**IR8**	**Ingredient**	**SR9**
Dapagliflozin	10	Saxagliptin	5
SSG	-		
Laycoat RS 720	-	Carbapol® 940	-
Ludiflash®	6	Karaya gum	-
PVP K30	20	HPMC K15M	90
MCC	104	PVP K30	20
Magnesium stearate	6	MCC	q.s.
Talc	4	Talc	4
Total	150	Magnesium stearate	6
		Total	150

Precision and accuracy

QC samples of known dapagliflozin concentrations (i.e., 30, 500, and 800 ng mL^{-1}) and saxagliptin concentrations (i.e., 15, 250, and 400 ng mL^{-1}) were used to evaluate the assay's precision and accuracy. Three days were needed to examine six replicates of each QC, and the required data was computed. By analysing triplicate (n = 6) samples, the recovery of dapagliflozin from plasma samples was performed at three concentration levels (LQC, MQC and HQC). Peak areas of actual analytes (in mobile phase) and QC samples in plasma were compared at the same final concentrations. The level of recovery of dapagliflozin, saxagliptin and the IS should be consistent, accurate and repeatable. The recovery was given as a percentage value.

Dapagliflozin and saxagliptin pharmacokinetic study

Healthy animals weighing 2.5-3 kg were chosen, housed in accordance with regulatory recommendations, fasted the previous night, and provided with unlimited access to drinking water. The results are shown in Table II.

Table II: *In vivo* data of optimized dapagliflozin and saxagliptin bilayered tablets

Time (h)	Dapagliflozin (ng mL^{-1})	Saxagliptin (ng mL^{-1})
0	0	0
0.5	28.85±9.62	16.84±11.68
1	35.59±3.59	28.86±16.94
1.5	41.36±14.56	25.96±8.54
2	52.87±9.84	24.63±9.68
2.5	66.63±6.55	23.69±12.54
3	72.69±14.29	21.59±18.26
4	85.26±9.87	20.38±11.54
6	96.28±6.99	19.18±10.39
8	109.36±8.78	16.63±21.54
10	129.84±9.28	14.38±18.26
12	144.62±6.51	11.98±9.54
16	88.26±3.66	9.63±10.26
20	57.28±5.10	7.62±3.55
24	32.22±2.88	5.14±10.24

Experimental design

Two experimental groups of six (n=6) each were created from the separated animals. Under fasting conditions, the following treatment regimen was used to compare the test formulation of batch (F) to the reference formulation. The results are shown in Table III.

Group I - Control formulation

Group II - Dapagliflozin and saxagliptin formulation (F) used as a test.

Animal dose calculation

Dapagliflozin

HED (mg kg^{-1}) = Animal dosage (mg kg^{-1}) X Animal km/Human km

10 mg/60 kg is the Human equivalent dose

Animal Km factor: 12; Human Km factor: 37; Rabbit weight: 2 kg

Table III: Statistical treatment of pharmacokinetic parameters (Mean±S.D.) of dapagliflozin and saxagliptin optimized formulations

Pharmacokinetic parameter	Optimized formulation of dapagliflozin	Optimized formulation of saxagliptin
Cmax	144.62±0.82	28.86±0.28
Tmax	12±0.16	1.00±0.05
AUC(0-t)	2046.478±2.04	322.3525±2.63
AUC(t-∞)	69.64413±2.51	66.0461±1.58
AUC(0-∞)	2116.122±3.95	388.3986±2.08
Kel	0.462638±0.02	0.077824±0.04

Therefore, the dose of the medication administered is 0.052 mg kg^{-1}.

Saxagliptin

HED (mg kg^{-1}) = Animal dosage (mg kg^{-1}) X Animal km/Human km

5 mg/60 kg Human equivalent dose

Animal Km factor: 12; Human Km factor: 37; Rabbit weight: 2 kg

Therefore, the dose of the medication administered is 0.026 mg kg^{-1}.

An oral gauge was used to administer the optimised formulation at a dose of 0.052 mg of dapagliflozin and 0.026

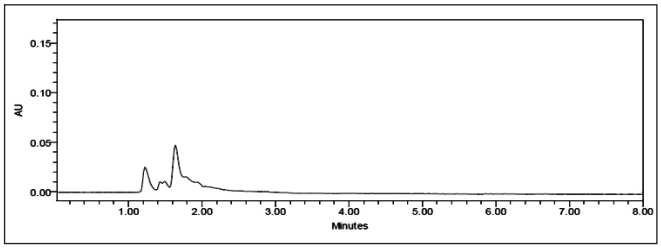

Fig. 1: Chromatogram of blank plasma

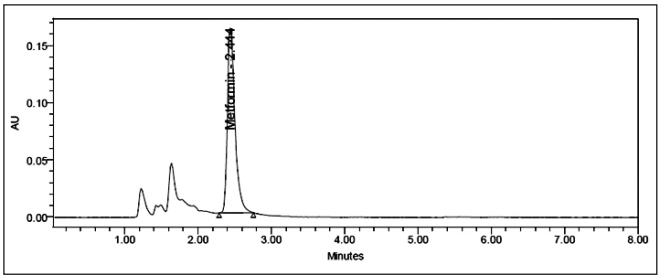

Fig. 2: Chromatogram of internal standard

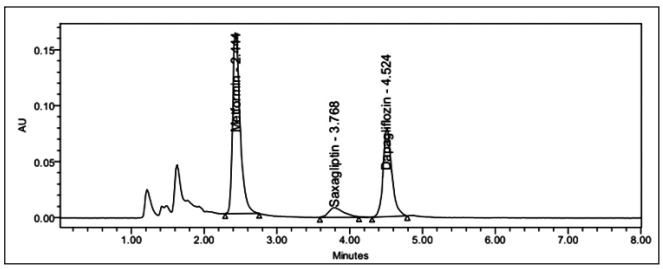

Fig. 3: Chromatogram of optimized formulation with internal standard in plasma

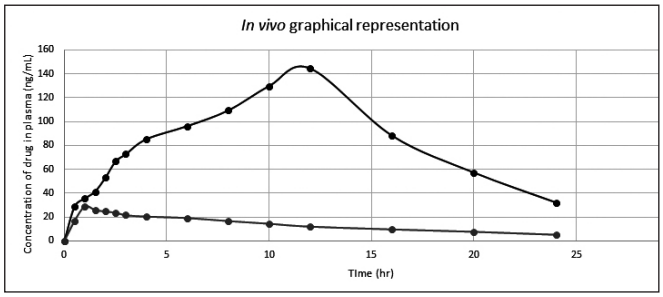

Fig. 4: Plasma concentration profile of marketed formulation

mg of saxagliptin. At regular intervals after administration, blood samples (each of around 1-2 mL from each animal) were taken from the marginal ear vein. The obtained blood samples were immediately spun for 10 minutes at 40 °C at 5000 rpm in an ultra-cooling centrifuge. In preparation for further analysis, the supernatant sample of plasma was isolated and kept in sterile 5 mL polypropylene plasma tubes, using deep freezer kept at -20°C.

Estimation of drug from rabbit plasma

To precipitate the proteins from the stored plasma samples, 500 µL of plasma was mixed with 1 mL of acetonitrile at room temperature. The samples were centrifuged at 10,000 rpm for 15 minutes after being vortexed for 15 minutes on a vortex mixer. Each sample was introduced on to the HPLC column.

Data analysis

The parameters for pharmacokinetic assessment were the $AUC_{0-\alpha}$, the Cmax, and Tmax. Directly derived from experimental plasma concentration versus time data were the Cmax and Tmax. The AUC_{0-24h}, which was determined using the trapezoidal rule, was added to obtain the $AUC_{0-\alpha}$. Data differences in average were compared using independent sample t tests or sample analysis of variance (one way analysis of variance)[14]. The 95% confidence interval was used to establish the difference's significance (P=0.05). The chromatograms and graphs are shown in Fig. 1 to Fig. 4.

RESULTS AND DISCUSSION

The *in vivo* tests were carried out in accordance with the earlier outlined protocol and process. Bioanalytical methods used for the quantification are strongly reliant on the examination and interpretation of pharmacokinetic data (plasma, urine, saliva, serum, etc). The creation of specialised and sensitive bioanalytical techniques is crucial for the quantitative assessment of medicines and their metabolites during pharmacokinetic studies of analytes.

The results of the statistical analysis were used to determine the pharmacokinetic parameters such as half-life, elimination rate constant and absorption rate constant and AUC from the plot of time versus plasma concentration. Dapagliflozin's maximum plasma concentration (Cmax) was 144.62±0.82 at 12h (Tmax) and AUC of 2116.122±3.95 ng mL^{-1} h^{-1}. Saxagliptin's maximum plasma concentration (Cmax) was 28.86±0.28 at 1h (Tmax) and AUC of 388.3986±2.08 ng $mL^{-1}h^{-1}$, according to the results of the administration of the optimised formulations. According to the findings, bilayer tablets that were developed exhibited superior bioavailability since they had a higher bioavailability. The longer plasma drug concentration duration and higher bioavailability were signs that the study's goal had been accomplished.

CONCLUSION

Diabetes patients are at an increased risk and it is crucial that new medications for the condition are developed. The current study examined the effectiveness of combining and dapagliflozin and saxagliptin in the form of bilayer tablets, where dapagliflozin is released in an immediate release and saxagliptin is released in a sustained release form. The outcomes supported the use of bilayered tablets to properly deliver medications for the diabetes treatment.

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