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Comparative *In Vivo* Evaluation of Marketed and Optimized Formulations of Teneligliptin and Metformin Bilayered Tablets

Rajani V[1]*, Rajendra Prasad Y[2] and Lakshmana Rao A[3]

[1]Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Andhra Pradesh, India

[2]College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India

[3]V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Andhra Pradesh, India

*Corresponding author: E-mail id: rajanivetapalem@gmail.com

Abstract

The combination of Metformin and Teneligliptin is an attractive approach for the management of type-2 diabetes because the two pharmacological approaches have different and potentially complementary targets. A novel bilayer tablet, consisting of an immediate release layer containing Teneligliptin (20 mg) and prolonged release layer containing Metformin (500 mg) was developed. *In vivo* studies were carried out in rabbits by using the optimised formulation as a test product and marketed formulation as a reference. Based on the *in vivo* performance, the developed bilayer tablets showed superior bioavailability than the marketed tablets. A simple, sensitive and selective HPLC method was developed for the simultaneous determination for Metformin and Teneligliptin in rabbit plasma using a novel sample extraction procedure. Method validation was carried out according to ICH guidelines in rabbit plasma in order to evaluate the method for selectivity, linearity of response, accuracy, precision, recovery and stability of analytes during processing and storage. The total area under plasma concentration time curve ($AUC_{0-\infty}$), the maximum plasma concentration (C_{max}), and time to reach the maximum plasma concentration (Tmax) were selected as parameters for pharmacokinetic evaluation. The C_{max} and Tmax were obtained directly from the experimental data of plasma concentration versus time. $AUC_{0-\infty}$ was obtained by adding the AUC_{0-24h}, which was calculated by the trapezoidal rule. The differences in average of data were compared by sample analysis of variance (one way analysis of variance) or independent sample t test. The significance of the difference was determined at 95% confident limit (P=0.05).

Keywords: Metformin, Teneligliptin, Bilayer Tablets, Formulation.

Introduction

Teneligliptin is a potent and selective inhibitor of dipeptidyl peptidase-IV (DPP-4), orally active, that improves glycemic control in patients with type 2 diabetes (T2DM) primarily by enhancing pancreatic (α and β) islet function. Thus Teneligliptin has been shown both to improve insulin secretion and to suppress the inappropriate glucagon secretion seen in patients with T2DM. Teneligliptin reduces HbA_{1c} when given as monotherapy, without weight gain and with minimal hypoglycemia, or in combination with the most commonly prescribed classes of oral hypoglycemic drugs: Metformin, a sulfonylurea, a thiazolidinedione, or insulin. Metformin, with a different mode of action not addressing β-cell dysfunction, has been used for about 50 years and still represents the universal first line therapy of all guidelines (1). However, given the multiple pathophysiological abnormalities in T2DM and the progressive nature of the disease, intensification of therapy with combinations is typically required over time.

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Recent guidelines imply that patients will require pharmacologic combinations much earlier to attain and sustain the increasingly stringent glycemic targets, with careful drug selection to avoid unwanted adverse events, especially hypoglycemia (2). The combination of Metformin and Teneligliptin offers advantages when compared to currently used combinations with additive efficacy and complimentary mechanisms of action, since it does not increase the risk of hypoglycemia and does not promote weight gain. Therefore, by specifically combining these agents in a single tablet, there is considerable potential to achieve better blood glucose control and to improve compliance to therapy (3). A novel bilayer tablet, consisting of an immediate release layer containing Teneligliptin (20 mg) and prolonged release layer containing Metformin (500 mg) was developed. An *in vivo* evaluation study conducted to ascertain pharmacokinetic parameters in rabbits by using the optimised formulation as a test product and marketed formulation as a reference. Based on the *in vivo* performance, the developed bilayer tablets showed superior bioavailability than the marketed formulation.

Materials and Methods

The *in vivo* study of the optimized formulations was performed as per the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Prior approval by Institutional animals ethics committee (Ref: P3/IAEC/2016/1/VVIPS/VR) was obtained for conduction of experiments. Marketed Tenelnat M (Natco Pharma) and optimized bilayer tablet, consisting of an immediate release layer containing Teneligliptin (20 mg) and prolonged release layer containing Metformin (500 mg) prepared in the laboratory conditions and chosen on the basis of *in vitro* release studies and stability conditions were chosen as dosage forms for administration.

Preparation of Bilayer Tablets (4,5):

a. Preparation of immediate release layer: Teneligliptin, Ludiflash, mannitol were weighed and co-sifted through sieve No. # 40 (ASTM), blended in a poly bag for 10 min, mixed well with PVP K-90 binder solution to make a damp mass. Later the damp mass was passed through sieve No. # 20 (ASTM), and dried in a hot air oven at 60°C for 1h. Finally the granules are lubricated with magnesium stearate by mixing in a poly bag, for additional 2-3 min; which is used as upper IR layer.

b. Preparation of sustained release layer: Metformin, HPMC K100M and MCC were weighed were co-sifted through sieve No. # 40 (ASTM), blended in a poly bag for 5 min and lubricated with magnesium stearate and aerosil by mixing in the same poly bag, for additional 2-3 min; which is used as lower SR layer. Composition of Teneligliptin (IR) layer and Metformin (SR) layer of bilayered tablets is given in Table 1.

Validation of the Bioanalytical Method (6):

Method validation was carried out according to ICH guidelines in rabbit plasma in order to evaluate the method for selectivity, linearity of response, accuracy, precision, recovery and stability of analytes during processing and storage.

Table 1: Composition of optimized teneligliptin and metformin bilayered tablets

Ingredients	Immediate Release Layer
Teneligliptin	20
Ludiflash	3
Starch	9
Mannitol	64
Pvp K-90	3
Mg.stearate	4
Total	100
Ingredients	Sustained release layer
Metformin	500
HPMCK100M	225
MCC	248
Aerosil	4.5
Mg.Stearate	22.5
Total	1000

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a. Selectivity: Selectivity was checked by injecting blank plasma samples from six different rabbits to confirm no interfering peaks around the retention time of both Metformin & Teneligliptin and IS.

b. Calibration, linearity and quality control samples (7): Calibration was constructed by calculating the peak area ratio of Metformin & Teneligliptin to that of IS. For the preparation of calibration standards, working solutions of Metformin & Teneligliptin (250 µL) and IS (500 µL) were added to blank plasma (0.25 mL) to obtain final concentrations of 65 ng/mL, 130 ng/mL, 195ng/mL, 1040 ng/mL, 1300 ng/mL, 1560 ng/mL, 2080 ng/mL and 2600 ng/mL of Metformin and 9.5 ng/mL, 19 ng/mL, 28.5 ng/mL, 152 ng/mL, 190 ng/mL, 228 ng/mL, 304 ng/mL and 380 ng/mL of Teneligliptin and directly inject 10 µL into HPLC. The quality control (QC) samples were prepared in a similar manner as the calibration standards at three different levels: low quality control (LQC), medium quality control (MQC) and high quality control (HQC).

c. Precision and accuracy (8): The precision and accuracy of the assay were determined using QC samples of known Metformin & Teneligliptin concentrations (i.e., LQC, MQC and HQC), which were processed freshly each validation day as described for calibration curve standards. Six replicates of each QC were analyzed on 3 days, and the intra- and inter- assay means, standard deviation (SD) and CV were calculated. The recovery of Metformin & Teneligliptin from plasma samples was carried out at three concentration levels (LQC, MQC and HQC) by analysis of replicate (n=6) samples. The peak area of QC samples in plasma was compared with peak area of actual analyte (in mobile phase) at the same final concentrations. The recovery was expressedas percentage value, and the extent of recovery of Metformin & Teneligliptin and of the IS should be consistent, precise and reproducible.

Metformin & Teneligliptin Pharmacokinetic Study (9):

Healthy rabbits (New Zealand Albino) of either sex weighing 2.5-3kg were selected and housed with CPCSEA guidelines, fasted over night and had free access to drinking water.

a. Experimental design: Animals were separated into two experimental groups, each group consisting of six animals (n=6). The test formulation of batch (F) was compared with (reference/marketed formulation) with the following treatment schedule under fasted condition:

Group I- Marketed formulation

Group II- Metformin & Teneligliptin formulation (F) used as test.

b. Animal dose calculation (10):

Metformin:

HED (mg/kg)=Animal dose (mg/kg) X Animal Km factor/Human Km factor.

HED: Human Equivalent Dose (500mg/60kg)

Animal Km factor=12

Human Km factor=37.

Wt. of the rabbits=3kg

So the dose of the drug taken is 8.33mg/kg.

Teneligliptin:

HED (mg/kg)=Animal dose (mg/kg) X Animal Km factor/Human Km factor.

HED: Human Equivalent Dose (20mg/60kg)

Animal Km factor =12

Human Km factor =37

Wt. of the rabbits=3kg

So the dose of the drug taken is 0.333 mg/kg.

The optimized formulation was administrated via oral gauge at a dose 8.333 mg/kg for Metformin & 0.333 mg/kg for Teneligliptin. Blood samples (each of about 1-2 mL from each animal) were withdrawn from marginal ear vein at regular time intervals after administration. The collected blood samples were immediately centrifuged at 5000rpm in ultra cooling centrifuge for 10min at 4^0C. The

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supernatant plasma sample was separated and stored in a clean screw capped 5ml polypropylene plasma tubes at -20℃ in a deep freezer, until further analysis.

c. Estimation of drug from rabbit plasma (11): The stored plasma samples were processed at room temperature, 500 µL of plasma was added to 1 mL of acetonitrile to precipitate the proteins. The samples were vortexed on vortex mixer for 15min, followed by centrifugation at 10000rpm for 15min. The respective samples were injected into the HPLC column.

d. Data analysis (12): The total area under plasma concentration time curve ($AUC_{0-\infty}$), the maximum plasma concentration (C_{max}), and time to reach the maximum plasma concentration (Tmax) were selected as parameters for pharmacokinetic evaluation. The C_{max} and Tmax were obtained directly from the experimental data of plasma concentration versus time. $AUC_{0-\infty}$ was obtained by adding the AUC_{0-24h}, which was calculated by the trapezoidal rule. The differences in average of data were compared by sample analysis of variance (one way analysis of variance) or independent sample t test. The significance of the difference was determined at 95% confident limit (P=0.05).

Results and Discussion

The *in vivo* experiments were conducted as per the protocol and procedure described earlier. Bioanalytical methods employed for the quantitative determination of drugs and their metabolites in biological matrix (plasma, urine, saliva, serum etc) play a significant role in evaluation and interpretation of pharmacokinetic data. For the successful conduct of pharmacokinetic study, the development of selective and sensitive bioanalytical methods plays an important role for the quantitative evaluation of drugs and their metabolites (analytes).

The HPLC method was highly sensitive and suitable for the detection of drug in plasma even in low concentrations and the respective chromatograms were shown in Figs 1, 2 & 3. Plasma concentrations of Teneligliptin and

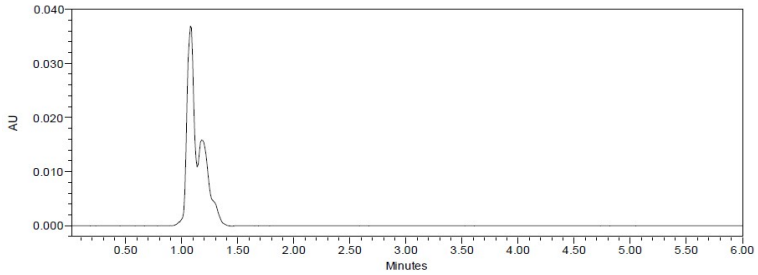

Fig 1. Chromatogram of blank plasma

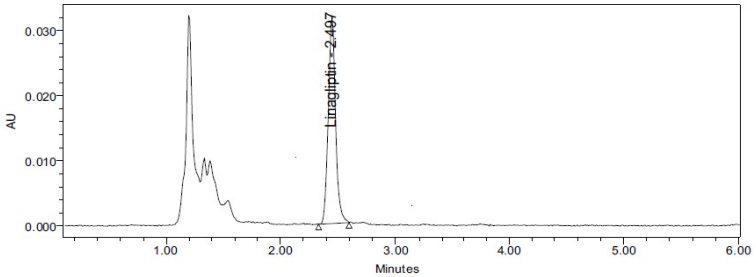

Fig 2. Chromatogram of internal standard

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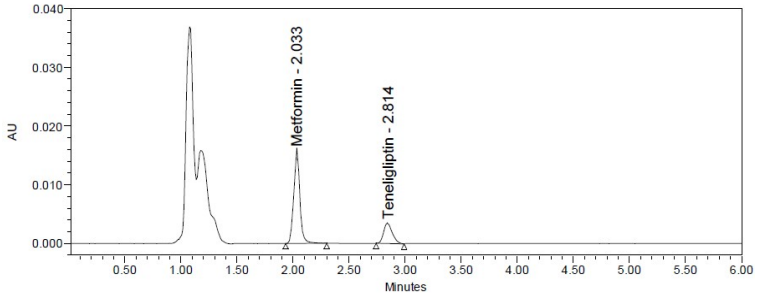

Fig 3. Chromatogram of optimized formulation with internal standard in plasma

Table 2. *In Vivo* data of metformin & teneligliptin in marketed formulation

Time (hrs)	Teneligliptin (ng/mL)	Metformin (ng/mL)
0	0	0
0.5	112.63±0.23	96.52±2.84
1	176.63±2.61	136.45±6.95
1.5	126.3±1.56	268.26±9.63
2	92.65±2.95	392.65±5.05
2.5	80.54±6.82	496.52±7.52
3	71.63±4.12	630.82±4.63
4	60.85±8.02	721.56±3.95
6	53.86±3.95	875.63±0.59
8	42.61±2.85	702.51±0.32
10	36.85±4.96	415.32±2.54
12	28.3±6.52	295.63±5.63
16	15.96±6.59	121.56±3.62
20	8.23±3.51	86.32±1.52
24	5.14±2.85	52.68±3.62

Table 3. *In Vivo* data of metformin & teneligliptin optimized formulation

Time (hrs)	Teneligliptin (ng/mL)	Metformin (ng/mL)
0	0	0
0.5	110.02±2.62	42.52±6.04
1	182.24±5.96	86.29±9.84
1.5	152.65±4.85	134.05±2.89
2	100.54±6.14	198.32±3.84
2.5	89.63±8.63	264.53±3.56
3	76.85±7.51	301.87±6.15
4	62.96±5.06	497.32±7.96
6	54.08±2.08	687.23±7.85
8	49.63±3.64	952.86±6.84
10	36.52±9.41	802.69±2.89
12	32.61±2.85	723.41±5.24
16	29.63±3.45	596.07±6.54
20	17.52±1.98	423.38±2.85
24	8.31±8.04	169.08±5.62

Metformin at different times were calculated and are shown in Table 2 & 3 and in Figs 4 & 5. Pharmacokinetic parameters such as absorption rate constant, elimination rate constant, half-life, AUC and MRT were calculated from the plot of time versus plasma concentration and subjected to statistical analysis and the results were shown in Table 4. The results from the oral administration of Teneligliptin from marketed formulation indicated the maximum plasma concentration (Cmax) 176.63±0.23 at 1hr (Tmax) while optimized formulations administration exhibited the maximum plasma concentration (Cmax) of 182.24±0.28 at 1hr (Tmax). The oral administration of marketed formulation resulted in a low and quite variable

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AUC of 925.368±2.85 ng/ml/hr, whereas the optimized tablets resulted in AUC of 1100.38±2.08 ng/ml/hr. The mean residence time of optimized tablets administration (4.8±0.09hrs) was found to be more than oral administration (4.3±0.06hrs). The results from the oral administration of Metformin from marketed formulation indicated the maximum plasma concentration (Cmax) 875.63±0.63 at

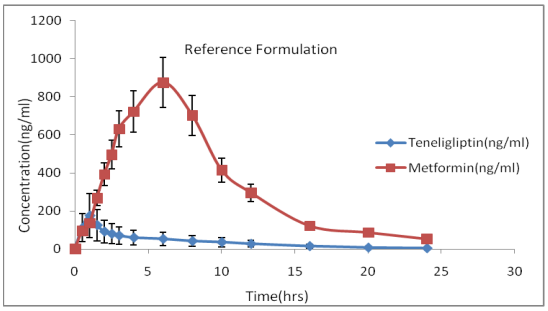

Fig 4. Plasma concentration profile of metformin & teneligliptin marketed formulation

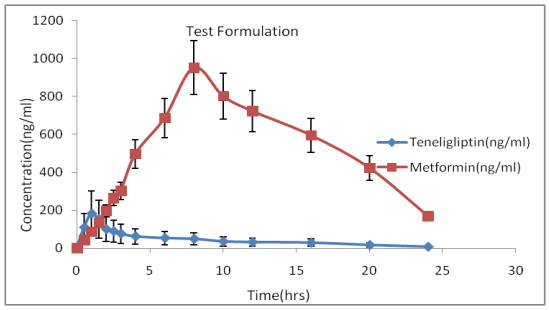

Fig 5. Plasma concentration profile of metformin & teneligliptin optimized formulation

Table 4. Statistical treatment of pharmacokinetic parameters (Mean±S.D.) of metformin and teneligliptin optimized formulation.

Pharmacokinetic Parameters	Optimized Formulation	Marketed Formulation	Calculated Value of 't'	Optimized Formulation	Marketed Formulation	Calculated Value of 't'-
	Teneligliptin	Teneligliptin		Metformin	Metformin	
Cmax	182.24±0.28	176.6±0.23	11.72***	952.8±0.82	875.63±0.63	16.14***
Tmax	1.00±0.05	1.00±0.65	01.13***	8.00±0.16	6.00±0.24	5.50***
AUC(0-t)	1046.1±2.63	890.9±1.54	40.75***	12696.7±2.04	7962.756±1.85	138.67***
AUC(t-∞)	54.2±1.58	34.4±2.63	18.87***	2312.7±2.51	516.90±1.04	47.66***
AUC(0-∞)	1100.3±2.08	925.3±2.85	19.67***	15009.4±3.95	8479.652±2.65	219.67***
Kel	0.159±0.04	0.143±0.01	26.60***	0.096±0.02	0.104±0.14	12.32***
MRT (h)	4.8±0.09	4.3±0.06	6.72***	7.1±0.04	6.6±0.04	17.11***
Null hypothesis (H_o): There is no significant difference between the pharmacokinetic parameters of marketed formulation and optimized formulations .Table value of 't' with 10 DF at the 0.001 level is 4.587.						
Result: H_o is not accepted as the calculated 't' value more than the table Value of't' with 10 DF at 0.001 levels of significance. It was therefore concluded that there was significant difference between the pharmacokinetic parameters of obtained with marketed formulation and optimized formulations.						

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6hr (Tmax) while optimized formulations administration exhibited the maximum plasma concentration (Cmax) of 952.86±0.82 at 8hr (Tmax). The oral administration of marketed formulation resulted in a low and quite variable AUC of 8479.652±2.65 ng/ml/hr, whereas the optimized tablets resulted in AUC of 15009.47±3.95ng/ml/hr. The mean residence time of optimized tablets administration (7.156±0.04hrs) was found to be more than oral administration (6.632±0.04hrs). Based on the results it was observed that greater bioavailability obtained from developed bilayer tablets showed superior bioavailability than the marketed tablets. The higher bioavailability and prolonged plasma drug concentration indicated that objective of this study was successfully achieved.

Conflict of interest

The authors declare that no conflict of interest.

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