VOLUME 9 ISSUE 2 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 2, 945-953 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Formulation Optimization and *In Vitro* and *In Vivo* Preclinical Evaluation of Vildagliptin Extended Release Tablets in Rabbits

Mallikarjun Naga P.[1, 2]* and Nagoji Eswara Venkata K.[3]

[1]Acharya Nagarjuna University, Guntur, Andhra Pradesh 522010, India
[2]Vignan institute of Pharmaceutical Technology, Visakhapatnam, Andhra Pradesh, India
[3]Sri Venkateswara College of Pharmacy, Etcherla, Srikakulam, Andhra Pradesh, India

**Corresponding Author*

Received: 2nd October, 2023; **Accepted:** 26th October, 2023; **Published online:** 2nd November, 2023

https://doi.org/10.33745/ijzi.2023.v09i02.106

Abstract: Vildagliptin, an oral antidiabetic medication commonly prescribed, has spurred a significant demand for extended-release tablet formulations. These formulations aim to provide a prolonged therapeutic effect while reducing the dosing frequency. The primary goal of this current research was to design and optimize extended-release Vildagliptin tablets, with a specific emphasis on harnessing the capabilities of natural gums, specifically *Lannea coromandelica* gum (LCG) and *Terminalia catappa* gum (TCG), to regulate the release of the drug.

The tablet formulation was prepared using the wet granulation method, and the optimization process was executed through a D-optimal mixture design employing Design Expert® software. The independent variables scrutinized were LCG (X1), TCG (X2), and lactose monohydrate (X3), with different combinations of gums. The dependent variables, or responses, under investigation included T50% (R1), T90% (R2), drug release at 2 h (R3), and mean dissolution time (MDT) (R4). The developed tablets underwent a thorough assessment of their physical properties and *in vitro* drug release profiles. To optimize the formulation, response surface plots were generated, and the ideal formulation was selected based on the highest desirability value. All the evaluated parameters across different formulations fell within the pharmacopoeial limits. The optimized tablet formulation exhibited physical properties well within the desired specifications and demonstrated an extended release profile, achieving 99% drug release over a 24 h period. Preclinical *in vivo* studies were conducted on rabbits, comparing pure drug suspension with the optimized vildagliptin formulation. *In vivo* studies also confirmed the extended release of the drug compared to the pure drug suspension.

The results suggest that matrix tablets of vildagliptin, using natural gums as release retardants, can be successfully developed, and these natural gums could serve as a promising platform technology for the development of extended-release matrix tablets for other drugs.

Keywords: D-optimal, *Lannea coromandelica* gum (LCG), *Terminalia catappa* gum (TCG), Vildagliptin, Extended-release

Citation: Mallikarjun Naga P. and Nagoji Eswara Venkata K.: Formulation optimization and *in vitro* and *in vivo* preclinical evaluation of vildagliptin extended release tablets in rabbits. Intern. J. Zool. Invest. 9(2): 945-953, 2023.

https://doi.org/10.33745/ijzi.2023.v09i02.106

Introduction

Vildagliptin belongs to a novel category of oral medications for managing diabetes and acts as a specific and reversible inhibitor of Dipeptidyl peptidase 4 (DPP-4), the enzyme responsible for deactivating incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These hormones play a significant role in regulating glucose levels. Vildagliptin can be used independently or in combination with other antidiabetic drugs. Following oral ingestion, it is quickly absorbed in the gastrointestinal tract (GIT). Vildagliptin has a biological half-life of 1.5 h. To ensure sustained and consistent blood levels over an extended duration and reduce the frequency of dosing, sustained-release formulations have been developed (Kolli *et al.*, 2014).

Type 2 diabetes mellitus (T2DM) is a intricate condition characterized by dysfunction in both islet cells, particularly beta- and alpha-cells, coupled with insulin resistance. Moreover, extensive clinical evidence, exemplified by findings from the UK Prospective Diabetes Study (UKPDS), highlights the progressive decline in beta-cell function in T2DM. This ongoing deterioration contributes to the perception of treatment inefficacy among T2DM patients and is the primary reason why many individuals with T2DM encounter difficulties in achieving target blood sugar levels. Additionally, the previously overlooked malfunction of alpha-cells in T2DM has now garnered recognition, especially in light of prior limitations in therapeutic options. The need to address this core deficiency in islet cell function has triggered a search for alternative treatments, leading to the rediscovery of incretin hormones and their role in regulating blood sugar levels. This enhanced understanding of their potential has subsequently fueled the development of incretin analogs and incretin enhancers for the management of T2DM (Chantal and Evy, 2008).

Currently, there are 11 medications within this classification designed for diabetes management. Although they all operate based on a common mechanism, they differ in their binding mechanisms, which in turn impact their therapeutic and pharmacological attributes. Dipeptidyl peptidase-4 inhibitors are seeing growing use because of their effectiveness and a lower risk of adverse effects when compared to the earlier generation of available drugs.

The D-optimal design represents a suitable approach for investigating and reducing the number of necessary experiments, examining the effects resulting from modifications in excipient mixture composition, and aiding in the selection of the most appropriate excipient composition for achieving an optimized formulation (Jin *et al.*, 2008). This method concentrates on incorporating all response variables into a quadratic model and, through regression analysis, establishes quantitative relationships between dependent variables (such as drug release rate) and independent factors like ingredient quantities, tablet hardness, and coating amounts. In this particular study, the D-optimal design employing a polynomial quadratic statistical model, as opposed to a simple quadratic model, was utilized, capitalizing on its acknowledged advantages. The study's objectives encompassed the application of the D-optimal design with a polynomial quadratic statistical model, in conjunction with contour surface response diagrams, to assess the influence of various independent formulation variables on drug release. This was conducted with the aim of optimizing an extended-release vildagliptin matrix tablet by comparing its dissolution profile (Vijayalakshmi *et al.*, 2008; Batul *et al.*, 2021).

Extended-release solid oral dosage forms, particularly hydrophilic matrices, are commonly employed due to their ability to achieve desired drug release profiles for a wide range of drugs. These matrices offer a robust formulation, cost-effective manufacturing, and enjoy broad regulatory acceptance of the polymers used (Tiwari and Rajabi-Siahboomi, 2008; Barhate *et al.*, 2015; Mondal *et al.*, 2018).

Gums, as a subset of hydrophilic matrices are utilized to attain extended-release profiles (Soumya *et al.*, 2014). In this study, relatively underexplored hydrophilic natural gums were used as release-retarding agents in the matrix tablets and to evaluate the tablets by *in vitro* and *in vivo* (pre-clinical studies).

Materials and Methods

Materials:

Vildagliptin was received as a gift sample from Lee Pharma, Visakhapatnam (India). *Lannea coramodelica* gum and *Terminalia catappa* gum were procured from local market. All other ingredients used were of analytical grade and procured from SD Fine-Chem Ltd., Mumbai (India).

Preparation of extended release matrix tablets of Vildagliptin :

Design of experiment:

A 3-factor 3-level D-optimal mixture design generated in Design Expert Software (version 10.0.6.0) was employed to study the effect of critical formulation on the product attributes of the extended release matrix tablets. The experimental design contained three factors or components, namely, the amounts of LCG (X1), TCG (X2), and Lactose (X3). The sum of three components would be 100% (194 mg) where the amount of X1, X2, and X3 were found to range from 25 mg to 150 mg, 35 mg to 160 mg, and 5 mg to 134 mg, respectively. The effect of formulation variables on responses like T50% (R1),T90% (R2), DR at 2 h (R3) and MDT (R4) was systematically investigated.

The extended-release matrix tablets of Vildagliptin, each containing 100 mg of the active ingredient, were produced through the wet granulation method. First, specific quantities of Vildagliptin, *Lannea coromandelica* gum (LCG), *Terminalia catappa gum* (TCG), and lactose monohydrate were passed through a 60-mesh sieve. These components were thoroughly mixed using the geometric dilution technique. After blending, granulation was achieved by employing an adequate amount of 5% polyvinyl pyrrolidone solution prepared in isopropyl alcohol (IPA). The resulting wet dough mass was then passed through a 12-mesh sieve, and the wet granules were subsequently dried in an oven at 60°C for 30 min. The dried granules were further sifted through a 24-mesh sieve. Lastly, the granules were enhanced with 1% magnesium stearate and 1% talc by blending them for 2-3 min. The lubricated mixture of the extended-release tablets was compressed into tablet form using a 9 mm circular die and punches on a rotary tablet compression machine (Elite) until a hardness of 5–7 kg/cm^2 was achieved. The formulation variables employed to produce 16 batches of extended release matrix tablets as per the experimental design are illustrated in Table 1.

Evaluation of the prepared tablets:

Tablets were evaluated for various quality control tests like the weight variation using electronic balance, hardness using monsanto hardness tester and % friability using Roche fabricator (Elite). The drug content of the tablets was also determined at pH 6.8 Phosphate buffer using UV spectrophotometer at 227 nm.

In vitro drug release study of optimization batches:

Drug release from extended release tablet was determined by using dissolution test (USP Type II) apparatus (LabIndia). *In vitro* dissolution study for first two hours was carried out in 900 ml of 0.1 N hydrochloric acid (pH 1.2) and then after 2 h, pH 1.2 buffer was replaced by pH 6.8 phosphate buffer for 22 h at 37±0.5ºC at 75 rpm. 10 ml of dissolution medium was withdrawn using a syringe fitted with 0.45 µm pre filter at regular time intervals and the same volume of fresh dissolution medium maintained at 37±0.5ºC was replaced and then the absorbance of the samples was measured at 227 nm using UV spectrophotometer (LabIndia). The cumulative % drug release was calculated for all the batches.

Prediction of the optimum formulation:

In order to obtain the optimized formulation, the numerical optimization technique was used and

Table 1: Formulation of optimization batches

Formulation	Vildagliptin	LCG	TCG	Lactose	Magnesium Stearate	Talc	Total weight
VLT1	100	81.65	60.4	51.95	3	3	300
VLT2	100	50.4	60.4	83.2	3	3	300
VLT3	100	50.4	91.65	51.95	3	3	300
VLT4	100	87.5	35	71.5	3	3	300
VLT5	100	25	97.5	71.5	3	3	300
VLT6	100	150	35	9	3	3	300
VLT7	100	89.5	99.5	5	3	3	300
VLT8	100	25	35	134	3	3	300
VLT9	100	112.9	61.4	19.7	3	3	300
VLT10	100	51.4	122.9	19.7	3	3	300
VLT11	100	25	35	134	3	3	300
VLT12	100	25	160	9	3	3	300
VLT13	100	25	160	9	3	3	300
VLT14	100	150	35	9	3	3	300
VLT15	100	87.5	35	71.5	3	3	300
VLT16	100	25	97.5	71.5	3	3	300

on the basis of the criteria set for dependent variables and the responses of the optimization batches the software gave three formulations with desirability close to 1. The formulation with maximum desirability was selected as the optimum formulation.

For validation three batches of selected optimum formula were prepared. The tablets were then evaluated for weight variation, friability, hardness, drug content and *in vitro* release profile. The comparison of the responses of the optimized batch suggested by the software with the response of the validation batches was done.

In vivo Study of optimization formulation:

The dose for each animal studies were calculated based on the guidelines given and reference from the U.S. Department of Health and Human Services Food and Drug Administration Centre for Drug Evaluation and Research (CDER) July 2005 Pharmacology and Toxicology for "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers"(FDA, 2005).

Human dose of vildagliptin = 100 mg for 60 kg

Human dose/kg = 100/60 = 1.67 mg/kg

Conversion factor for calculation of rabbit dose/kg=Human dose/0.32=5.21 mg/kg

Dose to be administered for an average rabbit weight of 2.6 kg = 13.6 mg

The following two products were tested for *in vivo* pharmacokinetic evaluation:

(i) Vildagliptin (13.6 mg) tablets (optimized formulation)

(ii) Vildagliptin pure drug suspension (13.6 mg)

As Vildagliptin studied is safe and for ease of its determination in plasma samples by HPLC, the products are tested in rabbits based on human doses (FDA, 2005) after approved by IAEC. Institutional Animal Ethics Committee (No. 2003/PO/Re/S/18/CPCSEA) approved the

Table 2: Observed response variables of optimization batches

Formulation	A:LCG	B:TCG	C:LACTOSE	T 50%	T 90%	DR at 2 h	MDT
				h	h	%	h
VLT1	81.65	60.4	51.95	8.62	15.52	24.3	6.99
VLT2	50.4	60.4	83.2	4.93	8.87	36.5	3.88
VLT3	50.4	91.65	51.95	8.64	15.55	26.5	7.07
VLT4	87.5	35	71.5	7.3	13.14	28.5	5.88
VLT5	25	97.5	71.5	7.2	12.96	29.2	5.59
VLT6	150	35	9	12.38	22.28	9.5	11.49
VLT7	89.5	99.5	5	11.92	21.46	9.8	10.94
VLT8	25	35	134	3.37	6.07	43.6	2.79
VLT9	112.9	61.4	19.7	11.78	21.21	14.2	10.62
VLT10	51.4	122.9	19.7	11.55	20.79	15.8	10.17
VLT11	25	35	134	3.38	6.08	45.3	2.76
VLT12	25	160	9	11.98	21.56	11.98	10.89
VLT13	25	160	9	11.86	21.35	12.28	10.75
VLT14	150	35	9	12.43	22.37	8.9	11.57
VLT15	87.5	35	71.5	7.33	13.2	27.3	5.9
VLT16	25	97.5	71.5	7.1	12.78	29.9	5.47

protocols. The *in vivo* study was conducted as per crossover RBD (n=6). Healthy rabbits weighing 2.0 - 2.6 Kg were used. The washout period was one month. After collecting the blank blood sample, the product in the study was administered orally with 10 ml of water. Blood samples (1.0 ml) were collected from marginal ear vein at different times (0.5, 1, 2, 4, 8, 12, 18 and 24 h) after administration. Samples were collected into heparinized test tubes and were centrifuged for 15 min at 10,000 rpm. The plasma samples were stored under refrigerated conditions at 4 - 8^{o}C prior to assay for drug content on the same day. The plasma concentrations of Vildagliptin were determined by HPLC method of Satheeshkumar *et al.* (2014) after revalidation.

Results and Discussion

Observations of optimization studies:

The hardness of various tablet formulation batches prepared in optimization studies (VLT1 to VLT16) was found to be 6 to 7 kg/cm^2 and friability of these respective tablet batches ranged from 0.57% and 0.89% (average 0.68%). The tablet weight of these optimization batches varied between 300.24 and 300.11 mg (average 300.10 mg) and the drug content was found between 99.76% and 101.21% (average 99.78%). The % cumulative drug release of the optimization batches was also studied over the period of 24 h and was found in the range. The observed responses are given in Table 2.

Response surface analysis for various responses:

The design was evaluated by a Scheffe's quadratic model. The equation representing the model is given below:

$$Y= \beta_1 X_1+\beta_2 X_2+\beta_3 X_3+\beta_{12} X_1 X_2+\beta_{13} X_1 X_3+\beta_{23} X_2 X_3$$

Where, Y is the measured response associated with each factor level combination, β_1, β_2 and β_3 are coefficients of the factors and β_{12}, β_{13} and β_{23} are coefficients of the interaction terms of factors.

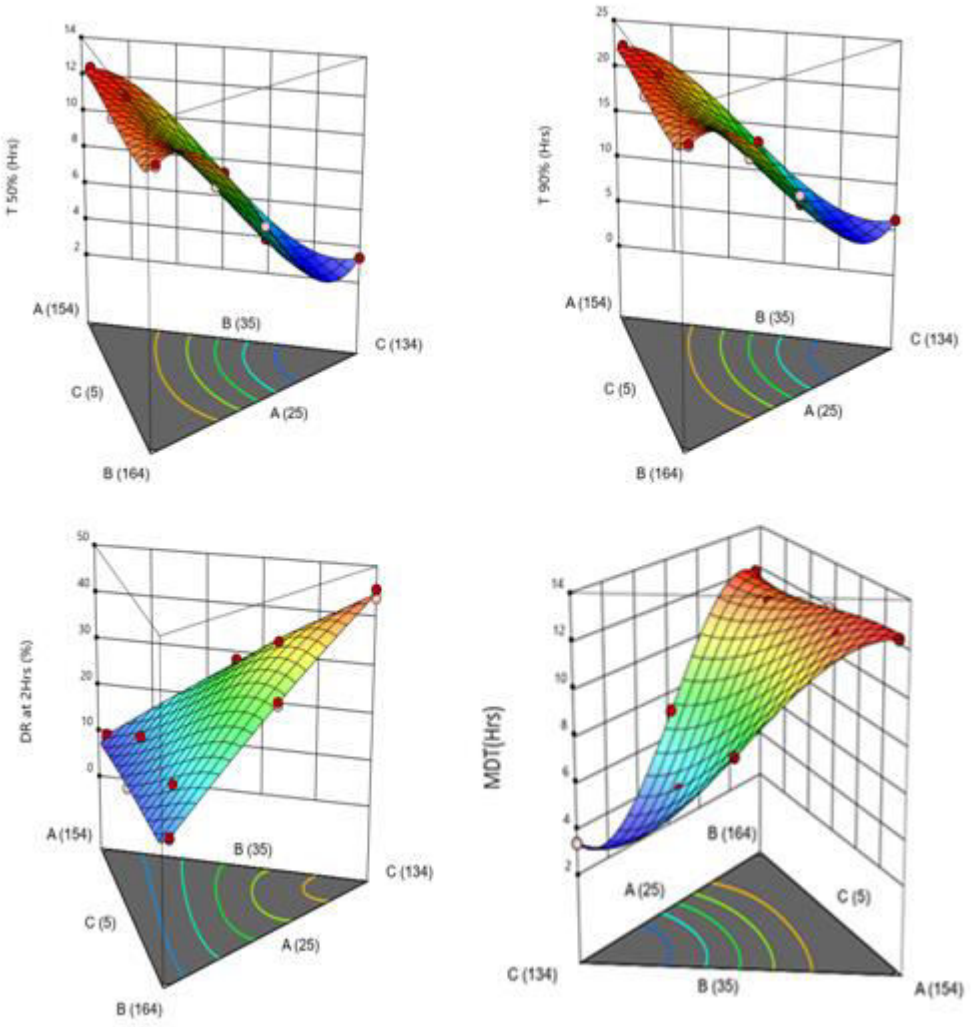

Fig.1: 3D surface plots of responses (A) T50% (B) T90% (C) DR at 2h (D)MDT Selection and validation of predicted optimum formulation.

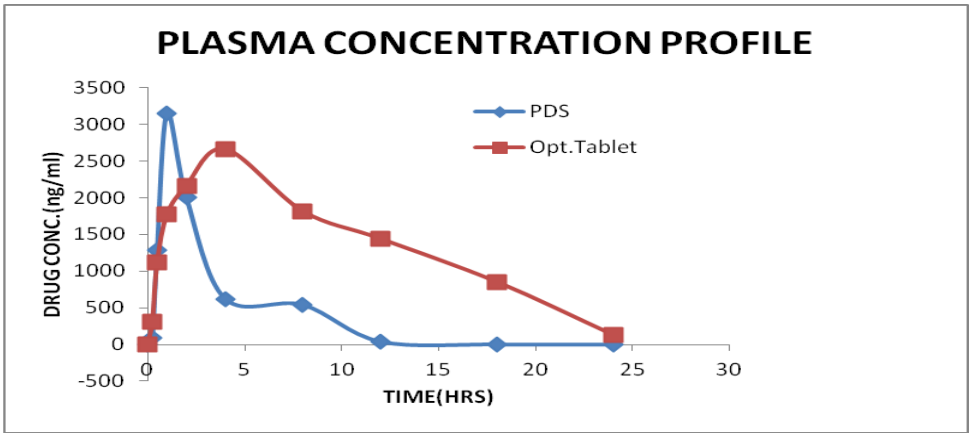

Fig. 2: Plasma concentration of Vildagliptin in Pure drug solution and Optimized formulation.

Table 4: Pharmacokinetic parameters

Pharmacokinetic Parameter	Units	Pure drug suspension	Optimized formulation
t1/2	h	1.934	4.963
Tmax	h	1.000	4.000
Cmax	ng/ml	3143.240	2663.693
AUC 0-t	ng/ml*h	9981.073	33048.817
AUC 0-inf_obs	ng/ml*h	10088.921	33983.278
AUC 0-t/0-inf_obs		0.989	0.973
AUMC 0-inf_obs	ng/ml*h^2	35916.918	315309.210
Frel of OPT.F relativeto PDS			331.114
MRT 0-inf_obs	h	3.560	9.278

Table 2 shows the observed responses of various optimization batches. The T50% ranged from 3.37 h to 12.43 h, T90% ranged from 6.07 h to 22.37 h, Drug release at 2 h ranged from 8.90% to 45.30% and MDT ranged from 2.76 h to 11.57 h.

All the responses were fitted into various models i.e., first order, second order, quadratic and cubic models and the model with highest regression coefficient was selected for the responses. On the basis of R^2 value amongst the responses, R1 and R2 followed Cubic model and R3 and R4 followed special quartic model. The effect of factors on the response variables are shown in 3D surface plots (Fig. 1).

Numerical optimization was conducted based on the specified criteria, as detailed in Table 1. The software provided seven potential solutions, from which the solution with the highest desirability score was chosen. The chosen formulation achieved a desirability score of 1.000 and included 138.68 mg of *Lannea coromandelica* gum (LCG), 50.319 mg of *Terminalia catappa* gum (TCG), and 5.000 mg of lactose. Subsequently, three batches of this optimized formulation were prepared and subjected to assessments of various physical parameters and predefined responses. The average measurements for tablet hardness, friability, tablet weight, and drug content in these validation batches were determined to be 6.5 kg/cm^2, 0.71%, 300.18 mg, and 99.17%, respectively. As a result, all physical parameters in the optimized validation batches were observed to be well within acceptable limits. The comparison of the responses, i.e., % drug release of the optimized batch suggested by the software with the responses of the validation batches, is shown in Table 3.

Table 3: Responses of the validation batches

	Predicted	Observed
T 50%	12.4049	11.8±1.2
T 90%	22.3237	22.3±1.1
DR at 2Hrs	8.68346	8.91±0.85
MDT	11.613	11.16±0.48

Preclinical Pharmacokinetic Evaluation:

Plasma concentrations of Valsartan observed following the oral administration of valsartsan IR tablets are shown in Figure 2. The pharmaco-kinetic parameters estimated are summarized in Table 4.

The peak plasma concentration (Cmax) of vildagliptin from Pure drug suspension and optimized formulation were found to be 3143.240 and 2663.693, respectively. The Cmax of optimized was found to be almost similar to PDS indicating the concentrations were lying in

therapeutic range. The Tmax values were found to be 1 h and 4 h for Pure drug suspension and optimized formulation respectively indicating that the drug was slowly releasing from optimized formulation in comparison to pure drug suspension. The release of drug in case of vildagliptin blood sample collected at 24 h time point showed higher plasma vildagliptin concentrations as compared with PDS. The t1/2 of vildagliptin from PDS and optimized were found to be 1.934 and 4.963, respectively.

The values of AUC0-24 of vildagliptin from PDS and optimised were found to be 9981.073 and 33048.817, respectively. The values of AUC0-∞ of vildagliptin from PDS and optimised were found to be 10088.921 and 33983.278, respectively. The relative bioavailability calculated relative to PDS based on AUC0-t for optimized formulation was 331.114. These values clearly indicated that the drug released from the matrix tablets prepared with the selected natural gums was extended.

The values of MRT0-∞ of vildagliptin from PDS and optimized were found to be 3.56 hours, 9.178 h and the MRT0-∞ values of vildagliptin were higher in the Optimized formulation due to slow release of drug.

Conclusion

The successful development of the extended-release Vildagliptin tablet formulation was achieved by incorporating *Lannea coromandelica* gum (LCG) and *Terminalia catappa* gum (TCG). To tailor the release characteristics of Vildagliptin from the tablet, a D-optimal mixture design was employed. The response surface plots facilitated an understanding of how these gums affect the drug release pattern. The validation of the optimization process included the creation of three distinct batches and an evaluation of their responses. The fitting of the release profile model revealed that the first-order model provided the best fit. Consequently, the use of natural gums like LCG and TCG can be harnessed to achieve the desired drug release pattern, and the optimization of these polymers for the desired drug release profile can be effectively accomplished using optimization techniques. The optimized tablet formulation developed for *in vivo* studies in animals also exhibited slow release and higher bioavailability (331.114%) when compared to Vildagliptin pure drug suspension.

Acknowledgements

The authors express sincere thanks to Dr.Y.Srinivasa Rao, Principal, Vignan Institute of Pharmaceutical Technology and Dr.L.Rathaiah, Chairman, Vignan Group of Institutions for providing necessary facilities to carry out this research work.

References

Barhate S and Hussain M. (2005) Development of hydrophilic matrix tablet of carbamazepine using 33 full factorial experimental designs. Int J Pharm Pharm Sci. 7: 369-375.

Batul S, Prakash K. Soni, Suresh K. Paswan and Saini T R. (2021) Formulation and optimization of natural gum based extended release tablets of Losartan using D-optimal mixture design. Int J Appl Pharm. 13(3): 214-220.

Chantal M and Evy D. (2008) Vildagliptin: a new oral treatment for type 2 diabetes mellitus. Vascular Health Risk Management 4(6) :1349-1360.

Jin X, Zhang Y, Xaio L and Zhao Z. (2008) Optimization of extended zero-order release gliclazide tablets using D-optimal mixture design. Yakugaku Zasshi 128: 1475-1483.

Kolli S, Dharavatu P, Sravani R, Srinivas Reddy K and Vinay Kumar D. (2014) Formulation and evaluation of vildagliptin sustained release matrix tablets. Int J Curr Pharmaceut Res. 6(4): 69-75.

Mondal N. (2018) The role of matrix tablet in drug delivery system. Int J Appl Pharm. 10: 1-6.

Satheeshkumar N, Pradeepkumar M, Shanthikumar S and Rao VJ. (2014) Development of validated stability indicating assay method for simultaneous estimation of metformin hydrochloride and vildagliptin by RP-HPLC. Drug Res (Stuttg) 64(3): 124-129.

Soumya P, Rao NGR, Kistaya C and Reddy BM. (2014) Design and development of oral sustained release matrix tablets of didanosine. Asian J Pharm Clin Res. 7 (Suppl) 1: 38-44.

Tiwari SB and Rajabi-Siahboomi AR. (2008) Extended-release oral drug delivery technologies: monolithic matrix systems. Methods Mol Biol. 437: 217-243.

US Department of Health and Human Services (2005) Guidance for Industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. Food and Drug Administration, Center for Drug Evaluation and Research, 2005.

Vijayalakshmi, P, Devi VK, Narendra C and Srinagesh S. (2008) Development of extended zero-order release gliclazide tablets by central composite design. Drug Develop Industrial Pharm. 34: 33-45.