Journal of Pharmaceutical Research International

33(1): 76-86, 2021; Article no.JPRI.64733
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Bioanalytical Method Development and Validation for the Simultaneous Determination of Vildagliptin and Telmisartan in Rabbit Plasma Using RP-HPLC

Budideti Kishore Kumar Reddy[1], Kothapalli Bonnoth Chandra Sekhar[2] and Chinnala Krishna Mohan[3*]

[1]Jawaharlal Nehru Technological University Anantapur, Ananthapuramu- 515002, Andhra Pradesh, India.
[2]Department of Chemistry, Jawaharlal Nehru Technological University Anantapur, Ananthapuramu-515002, Andhra Pradesh, India.
[3]Department of Pharmacology, School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad- 500088, Telangana, India.

Authors' contributions

The work was carried out in collaboration among all authors. Author BKKR designed the study, wrote the protocol, performed the Experiment and wrote the first draft of the manuscript. Author KBCS Proof reading of the manuscript and finalized the manuscript. Author CKM managed the literature searches and performed the statistical analysis. All the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i131141
Editor(s):
(1) Dr. Q. Ping Dou, Wayne State University, USA.
Reviewers:
(1) Rogério Rodrigo Ramos, Universidade Brasil, Brazil.
(2) Ghada Abd Alrhman Taqa, University of Mosul, Iraq.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64733

Original Research Article

Received 25 November 2020
Accepted 30 January 2021
Published 13 February 2021

ABSTRACT

A simple, reproducible bioanalytical method of liquid chromatography and PDA detector was developed and validated for the simultaneous Determination of Vildagliptin and Telmisartan in Rabbit Plasma using liquid-liquid extraction technique. K2 EDTA was used as anti-coagulant. Analytes were extracted by Methyl-tert-Butyl Ether (MTBE) and subsequent separation on a Kromasil C18 column (5 µ, 100 × 4.6 mm) using Acetonitrile : Methanol 75:25 v/v as mobile phase at a flow rate of 1 mL/min and (40±1)°C column oven temperature. Analytes were monitored with PDA detector at an isosbestic point of 225 nm for both Vildagliptin and Telmisartan. Retention times of Vildagliptin and Telmisartan were found to be at 2.545 mins and 6.633 mins respectively.

**Corresponding author: E-mail: bkkreddypharma@gmail.com, kishore.pharmacy@jntua.ac.in;*

The method was validated over a linear (r^2 = 0.9979) concentration range of 24.979 - 5003.808 µg/ml for Vildagliptin and 1.011- 202.559 µg/ml for Telmisartan. The inter-day and intra-day precisions were found to be less than 15% and the accuracy was all within ±15% (at LLOQ ±20%). The developed HPLC-PDA method was fully validated for all the other parameters as per FDA guidelines like selectivity, matrix effect, recovery and stability as well. Due to the high degree of sensitivity, very less time consuming, easy extraction procedure and low requirement of sample volume, the method will be applicable for therapeutic drug monitoring.

Keywords: Bioanalytical; liquid chromatography; vildagliptin; telmisartan, rabbit plasma.

1. INTRODUCTION

Vildagliptin (VIL), *S*-1- [*N*- (3 - hydroxyl - 1 - adamantyl) glycyl] pyrrolidine - 2 - carbonitrile (Fig. 1), is an oral hypoglycemic drug of the dipeptidylpeptidase-4 (DPP-4) inhibitor class [1,2]. DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes [3]. Telmisartan (TEL) is 2-[4-[[4-methyl-6-(1- methylbenzimidazol-2-yl)-2 propylbenzimidazol-1-yl]-methyl]phenyl] benzoic acid (Fig. 2), is a synthetic analogue of angiotensin II receptor blocker, used for the treatment of hypertension. TEL is a poorly water-soluble drug which displays a dissolution rate-limited absorption pattern in humans and animals. Hence, it can be used as a model drug to assess the influence of various physicochemical, physiological, and dosage form factors on the absorption kinetics and bioavailability of hydrophobic drugs [4-7]. Chemical structures of VIL and TEL were shown in Figs. 1 and 2 respectively.

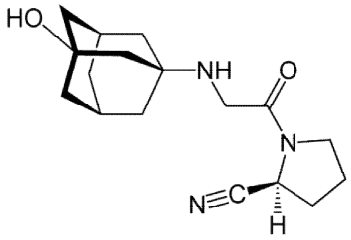

Fig. 1. Structure of Vildagliptin

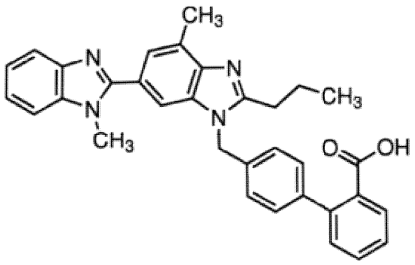

Fig. 2. Structure of Telmisartan

Literature survey reveals that vildagliptin can be estimated by UV spectroscopic method [8], RP-HPLC method, which is a time consuming method being the retention time is more than 10 min [9], RP-LC/MS method, requires mass spectroscopy detection [10]. Few other methods for the quantitative estimation of VIL separately and in combination with other drugs were reported [11-14]. A variety of methods have been developed for determination of TEL individually or with combination with some other antihypertensive agents in biological samples [15-17]. This includes, HPLC coupled with mass spectrometric (HPLC–MS) and spectrofluorimetric detection. [18,19] In general, spectrofluorimetric method lack sensitivity and cannot distinguish degradation products from the parent compound. Although HPLC–MS methods provide excellent sensitivity, they are not available in all laboratories because of their special requirements and economic considerations. Moreover, spectrofluorimetric method utilized either a column switching system or an expensive solid phase extraction cartridges.

With respect to these, all reported methods for the determination of VIL and TEL have various limitations: time-consuming sample clean-up, laborious extraction steps and long chromatographic elution time. Moreover, to the best of author's knowledge no bioanalytical method was reported for simultaneous estimation of VIL and TEL in rabbit plasma. Thus, the present study has been undertaken to develop and validate a simple, sensitive, accurate, precise and reproducible bioanalytical HPLC-PDA method for estimation of the simultaneous estimation of VIL and TEL in rabbit plasma.

The bioanalytical methods used for the quantification of drugs in biological samples play a very important role in the evaluation and interpretation of bioavailability and bioequivalence data. Therefore, complete validation of the analytical method was

performed in accordance with USFDA guidelines [20] to obtain reliable results that could be interpreted satisfactorily.

2. MATERIALS AND METHODS

2.1 HPLC–PDA Instrumentation and Chromatographic Conditions

The HPLC system was an C Waters (Waters, Milford, MA, USA) consisting of quaternary gradient system (600 Controller), in line degasser (Waters, model AF), photodiode array detector (Water, 2998 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower Pro software (Waters, Milford, MA, USA). Chromatographic separation assay was performed with a Kromasil C18 column (5µ, 100 × 4.6 mm) maintained at ambient temperature. The mobile phase consists of Acetonitrile: Methanol 75:25 v/v. The mobile phase was pumped at a flow rate of 1.0 mL/min. The detection wavelength was 225 nm. Mobile phase was used as diluent for the preparation of working standards of VIL and TEL. Injection volume was 10µl. The run time was 9 mins and the retention time of VIL and TEL was found to be 2.545 min and 6.633 mins respectively.

2.2 Chemicals and Reagents

The reference sample of VIL (99.99%) and TEL (99.98%) was gifted by Cadila Health Care Ltd., Ahmadabad, India. HPLC type II water from Millipore's Milli-Q system was used throughout the analysis. HPLC grade Methanol, Acetonitrile and Methyl-tert-Butyl Ether (MTBE) were purchased from Merck, Mumbai, India. All other chemicals were of analytical grade purchased from SD Fine Chem, Mumbai, India.

2.3 Animals and Ethical Committee

This study was conducted on four groups of animals, each group contains six healthy white male albino rabbits which were purchased from animal house of Adita Biosys Private Limited (1868/PO/BT/S/16/CPCSEA), Bangalore, with average body weight of 1250 ± 50 g. Rabbits were chosen due to larger volume of blood was required to perform Pharmacokinetic study and other parameters of study. Animals were kept under prerequisite temperature of $(23 \pm 2)\ ^{0}$C with humidity 50–60% in a light-dark cycle of 12 h each. Both control and tested animals were kept in same environment and provided with the standard food. Steel bottom cages were used to keep each rabbit separately with free access of food and water.

2.4 Blood Samples Collections

The blood samples were collected from the marginal ear vein of normal healthy rabbits at the time intervals of 0, 1, 2, 4, 6, 8, 10, 12 and 14 hours (H) respectively after dosing.

2.5 Preparation of Stock and Working Solutions

The stock solution was prepared by dissolving 25 mg of VIL in 25 mL methanol. This solution was further diluted in the same solvent to get 25 µg/mL of VIL. Similarly TEL was prepared at 36 µg / ml. Calibration standards were prepared by diluting the working standard solution of VIL to get the final concentrations of 24.979, 49.958, 249.790, 499.580, 1248.950, 2497.901, 4003.046, 5003.808 µg / ml. For TEL 1.011, 2.022, 10.112, 20.223, 50.559, 101.117, 162.047 and 202.559 µg / ml. Working solutions for quality control samples as: High quality control (HQC): 153.547 µg/mL, Medium quality control (MQC) 1: 75.125 µg/mL; MQC2: 2.810 µg/mL and Low quality control (LQC): 0.921 µg/mL. All solutions were stored at 2-8°C.

2.6 Sample Preparation

A volume of 200 µl of plasma was transferred into a vial, then 50 µl of VIL (25 µg / ml) and 50 µl of TEL (36 µg / ml) were added and 100 µl of 2% formic acid were added and stirred for 10 minutes. 2.5 ml of methyl tert-butyl ether were added and centrifuged at 2500 RPM for 10 minutes, the organic layer was transferred to a new vial and evaporated to dryness under a light stream of gaseous nitrogen at 45°C. The residue was reconstituted with 500 µl of diluent and 10 µL aliquots were injected into the HPLC system.

2.7 Quantification

VIL and TEL were determinate quantitatively using an eight-point calibration curve which was established in plasma using peak area ratio of analytes. The unknown concentration of samples and QC's were calculated by interpolation from the calibration curve.

2.8 Method Validation

Method validation protocol was prepared based on the guidelines of the United States Food and Drug Administration (USFDA) [20].

2.8.1 Calibration curve and lower limit of quantitation (LLOQ)

A series of eight calibration curve standards, were prepared to assess the linearity of the method. During the course of validation, each calibration curve was analyzed by using a $1/x^2$ weighted least square regression analysis of standard plots associated with an eight-point standard curve. The standard curve was chosen to cover the range of clinically significant concentrations of patients. The curve was found to be validated; at least 6 of 8 calibration standards should be less than 15% of the coefficient of variation (CV). A Correlation of more than 0.99 shall be desirable for all the calibration curves. The lowest concentration on the calibration curve was considered as the Lower limit of quantitation. The error of accuracy and CV should be less than 15% for all calibration standards and less than 20% for LLOQ. The response of LLOQ of the analyte should be at least 5 times higher than the response of in blank.

2.8.2 Precision and accuracy

To measure reproducibility precision and repeatability are significant factors. Precision and accuracy of the method were developed by analyzing six replicate samples of each LLOQ, low, mid (1 &2) and high quality control samples. To determine intraday precision and accuracy, the plasma samples were analyzed on the same day. The interday precision and accuracy were assessed by performing precision and accuracy batches on different days. Accuracy was measured as the percentage difference between the theoretical and the measured value according to the equation:

> Accuracy (%) = ((Measured Concentration - Theoretical Concentation) / (Theoretical Concentation)) X100%

The % deviation of each concentration level from the nominal concentration in the accuracy and precision must be less than 15% for all except for LLOQ, for which it should not be more than 20%.

2.8.3 Recovery from plasma

The extraction efficiency (recovery) of VIL and TEL were measured as the ratio between the mean analyte concentrations in plasma following LLE of QC samples of High quality control (HQC): 153.547 µg/mL, Medium quality control (MQC) 1: 75.125 µg/mL, (MQC) 2: 2.810 µg/mL; and Low quality control (LQC): 2.81 µg/mL to the equivalent concentrations dissolved directly in elution solution is recovery. As per the FDA guidelines [20], the recovery of the analyte need not be 100 %, but the extent of recovery of an analyte should be consistent, precise and reproducible.

3. RESULTS AND DISCUSSION

3.1 Optimization of Sample Preparation and Chromatographic Conditions

It is very essentials to adjust or tune the UV and chromatographic conditions as well, for the optimum detection and quantification of the analytes (VIL and TEL) in rabbit plasma VIL and TEL both the analytes showed good response and prominent peaks. Sample preparation is one of the key fundamental steps in the development of the bioanalytical method. Sample preparation must be fast, simple and easy to proceed and get the maximum recovery of analytes with a minimum amount of reagents and solvents. Literature review revealed the use of solid phase extraction (SPE) technique for the extraction of VIL. However, SPE is time consuming and expensive technique when compared to LLE method. Hence we used the LLE method for the sample preparation to cut the cost and shorten the processing time and acquire desired recoveries of the analyte. Some methods were reported to use higher plasma volumes for sample preparation and injection volume for the chromatographic development. Whereas the current method was developed with less plasma volume and injection volume, leads to better acceptability of the method. In order to get maximum recovery, a wide variety of extraction solvents and buffers were used to extract analyte from human plasma such as Diethyl Ether, Ethyl acetate, Methyl-tert-Butyl Ether: n-Hexane (80:20), Methyl-tert-Butyl Ether, etc. better recovery and response were obtained with Methyl-tert-Butyl Ether. There was no interference from any exogenous and endogenous plasma matrix.

In order to achieve good sensitivity, peak shape, and symmetry as well as short chromatographic run time for both analyte all chromatographic conditions were adjusted and optimized. In this study thermo, Gemini, symmetry, waters and luna columns with various mobile phases such as acetonitrile, methanol, formic acid, ammonium formate, ammonium acetate, phosphate buffers, and aqueous ammonia, etc were tired. Finally, Kromasil C18 column (5µ, 100 × 4.6 mm) using

Acetonitrile : Methanol (75:25 v/v) as mobile phase at a flow rate of 1 mL/min and 40 ± 1°C column oven temperature were selected because of better separation and detection. Due to low injection volume of 10 µL reduced overloading of the column with analytes, thereby ensuring more number of analyses on the same column. Finally, VIL and TEL were eluted at 2.545 min and 6.633 mins respectively. Figs. 3-5 represents the chromatograms of HQC (Fig. 3), MQC (Fig. 4) and LQC (Fig. 5).

3.2 Selectivity

There was no interference peaks observed due to endogenous or exogenous components at a retention time of the sample of VIL and TEL, extracted from rabbit plasma as represented in Figs. 3-5. The response of drug in blank plasma was less 2%. Typical retention times of VLD and TEL was 2.545 min and 6.633 mins respectively.

3.3 Calibration Curve

Calibration curves were found to be linear over the range of for VIL 24.979, 49.958, 249.790, 499.580, 1248.950, 2497.901, 4003.046, 5003.808 µg / ml. For TEL 1.011, 2.022, 10.112, 20.223, 50.559, 101.117, 162.047 and 202.559 µg / ml. The coefficient of correlation was found to be better than 0.99 for all the six calibration curves analyzed. The present bioanalytical method provided a lower limit of quantitation and a good range of linearity were shown in Table 1 exhibits the mean concentrations obtained for the calibration curve.

3.4 Precision and Accuracy

The intraday accuracy of the method was between 88.41 to 97.90 % with a precision of 0.41 - 12.23 % for VIL. The inter-day accuracy was between 93.01 - 107.55 % with a %CV of 2.87 - 6.63% for TEL. The data indicate that the method possessed adequate repeatability and reproducibility. Table 2 shows the data on precision and accuracy.

3.5 Recovery

The percentage recovery was determined by comparing the average area of the peak in samples taken with fresh non-extracted samples prepared in three concentrations. The % of average recoveries was determined by measuring the concentrations of the plasma quality control samples extracted in HQC, MQC1, MQC2 and LQC compared to the quality control samples extracted in HQC, MQC1, MQC2 and LQC. Results of recovery of VIL and TEL were shown in Tables 3 and 4 respectively. It has been documented that recovery% must be 80% in analytical methods. While the development of the bioanalytical method, the purpose of recovery, is not considered a problem as long as the method produces sensitivity, Accuracy and precision.

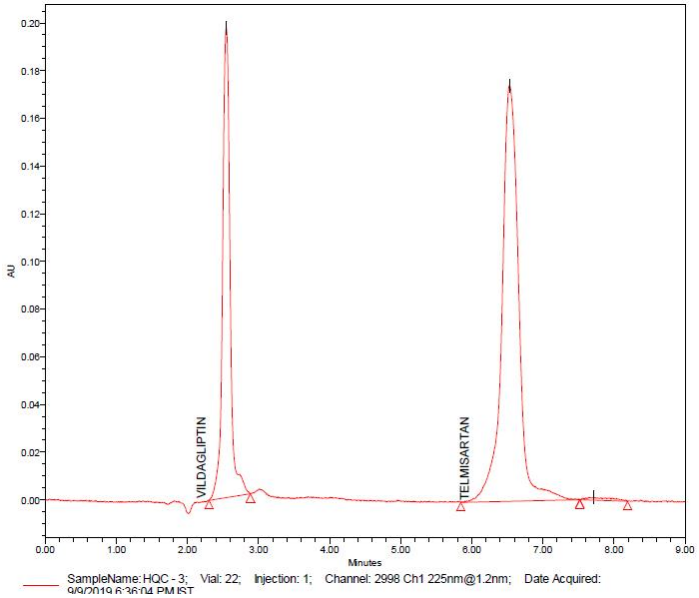

Fig. 3. Chromatograms of HQC at 153.547µg/ml

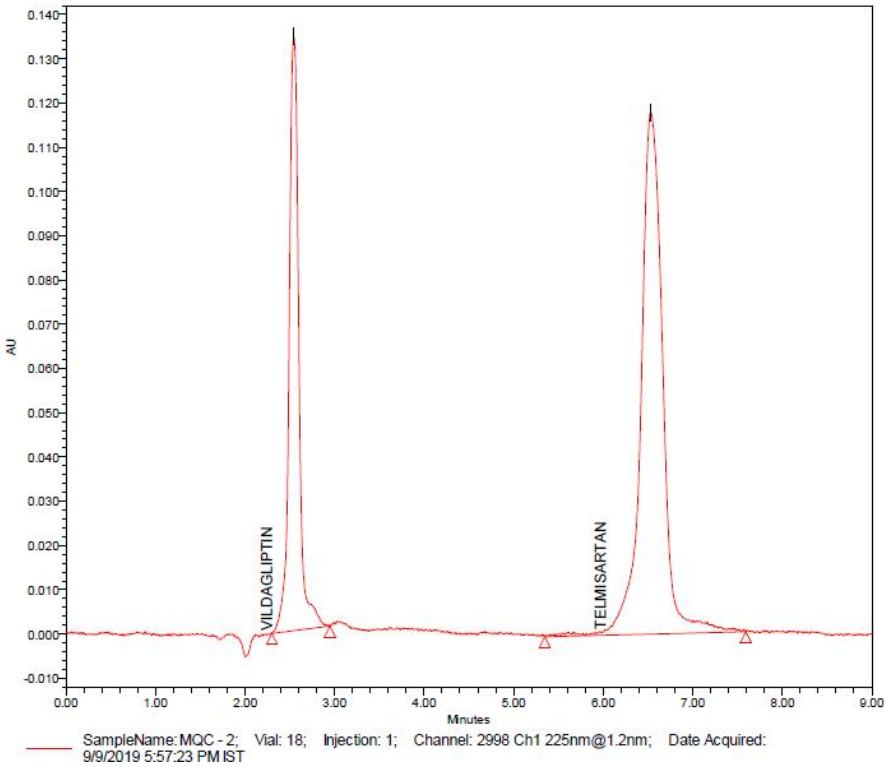

Fig. 4. Chromatograms of MQC at 75.125 µg/ml

Table 1. Calibration curve data with slope, intercept, correlation-coefficient (r^2) for VIL and TEL

S. No	VIL		TEL	
	Conc.(µg / ml)	Conc.	Conc.(µg / ml)	Peak area
1.	24.979	66898	1.011	1044966
2.	49.958	319312	2.022	1006410
3.	249.790	1071726	10.112	1378545
4.	499.580	1524140	20.223	1665003
5.	1248.950	2976554	50.559	2217317
6.	2497.901	5428968	101.117	3313437
7.	4003.046	7981382	162.047	4623766
8.	5003.808	9893796	202.559	5434096
Slope	1917		2164	
Intercept	40284		1E+06	
r^2	0.996		0.997	

3.6 Application to a Pharmacokinetic Study

Overlay graphs of mean concentration versus time of the two drugs are shown in Fig. 6. The area under the curve from 0 to 12 h was determined with the help of the linear trapezoidal rule. The extrapolation to infinity that is necessary for $AUC_{0-\infty}$was calculated using a linear regression model from the last three data points in the elimination phase that has been log-transformed. Maximum concentration achieved (C_{max}) was obtained directly from measured concentration without interpolation. The parametric point estimates for the mean of test medication were found within the commonly accepted bioequivalence range of 0.8-1.25. Therefore, the results indicate that the proposed method is suitable for pharmacokinetic studies to determine the concentration of Vildagliptin and Telmisartan in rabbit plasma. The study was conducted strictly in accordance with guidelines laid down by the International Conference on Harmonization (ICH) and USFDA. The pharmacokinetic data are tabulated in Table 5.

Table 2. Precision and accuracy data

Analytes	Vildagliptin				Telmisartan			
QC ID	**HQC**	**MQC**	**LQC**	**LLOQ QC**	**HQC**	**MQC**	**LQC**	**LLOQ QC**
Nominal Conentration (µg/mL)	164.918	82.459	2.866	1.032	153.547	75.125	2.810	0.921
				Calculated Concentration (µg/mL)				
P & A	159.240	77.826	2.985	1.139	151.806	76.292	2.527	0.794
	155.971	82.117	2.850	1.348	156.312	70.073	2.442	0.909
	170.093	83.202	2.754	1.114	144.294	72.569	2.491	0.774
	153.482	79.289	2.902	1.084	140.600	67.662	2.467	0.839
	162.336	78.705	2.728	0.968	146.092	72.515	2.530	0.909
	163.076	77.293	2.725	1.020	139.679	68.833	2.501	0.893
Mean	160.6997	79.7387	2.8240	1.1122	148.7752	72.3727	2.5445	0.8577
SD	5.88464	2.39004	0.10639	0.13146	6.04771	3.17724	0.13445	0.06432
% CV	3.66	3.00	3.77	11.82	4.06	4.39	5.28	7.50
% Mean Accuracy	97.44	96.70	98.53	107.77	96.89	96.34	90.55	93.12
P & A II	163.240	80.567	2.985	1.031	150.806	76.292	2.679	0.894
	159.203	81.214	2.171	0.998	156.312	74.073	2.791	0.909
	161.093	82.102	2.504	1.071	144.294	70.569	2.691	0.892
	159.258	80.251	2.618	1.100	147.600	77.662	2.467	0.839
	159.789	81.111	2.821	0.991	146.092	72.515	2.678	0.909
	164.025	79.935	2.678	1.123	142.679	69.833	2.802	0.913
Mean	161.1013	80.8633	2.6295	1.0523	149.7752	74.3727	2.6860	0.8940
SD	2.09024	0.78015	0.27982	0.05438	4.61215	2.57209	0.12207	0.02901
% CV	1.30	0.96	10.64	5.17	3.08	3.46	4.54	3.25
% Mean Accuracy	97.69	98.06	91.75	101.97	97.54	99.00	95.59	97.07
P & A III	164.011	82.201	2.785	1.105	153.658	73.987	2.510	0.900
	159.098	81.358	2.674	0.989	149.987	75.100	2.900	0.897
	163.987	82.037	2.524	1.001	150.301	74.824	2.799	0.919
	162.627	81.392	2.871	1.112	152.098	76.984	2.617	0.917
	163.701	80.990	2.799	0.981	153.001	77.515	2.800	0.899
	164.921	79.098	2.698	1.003	152.880	77.279	2.699	0.902
Mean	163.0575	81.1793	2.7252	1.0318	152.0987	75.5892	2.7393	0.9088
SD	2.07474	1.11574	0.12176	0.05997	1.61457	1.36100	0.14533	0.01125
% CV	1.27	1.37	4.47	5.81	1.06	1.80	5.31	1.24
% Mean Accuracy	98.87	98.45	95.09	99.98	99.06	100.62	97.49	98.68

Table 3. Recovery data of VIL

Replicate No.	HQC		MQC-1		MQC-2		LQC	
	Aqueous Response	Extracted Response	Aqueous Response	Extracted Response	Aqueous Response	Extracted Response	Aqueous Response	Extracted Response
1	44844	45784	25009	23430	8889	9290	887	811
2	44341	45512	25143	23661	8821	10000	836	891
3	44116	50116	25101	23612	8403	9864	875	759
Mean	44434	47137.3	25084.3	23567.7	8704.3	9718.0	866.0	820.3
SD	372.70	2583.20	68.50	121.70	263.20	376.80	26.70	66.50
% CV	0.84	5.48	0.27	0.52	3.02	3.88	3.08	8.11
% Mean Recovery	106.08		93.95		111.65		94.72	
Overall % Mean Recovery	101.600							
Overall SD	8.697							
Overall % CV	8.56							

Table 4. Recovery data of TEL

Replicate No.	HQC		MQC-1		MQC-2		LQC	
	Aqueous Response	Extracted Response	Aqueous Response	Extracted Response	Aqueous Response	Extracted Response	Aqueous Response	Extracted Response
1	9808	8239	10259	7442	9803	7716	10235	7340
2	9876	7897	9921	7730	9925	8088	10366	8015
3	9672	8528	10124	7105	9890	7786	10124	7286
Mean	9785	8221	10101	7426	9873	7863	10242	7547
SD	103.872	315.871	170.136	312.820	62.820	197.690	121.138	406.198
% CV	1.062	3.842	1.684	4.213	0.636	2.514	1.183	5.382
% Mean Recovery	104.25		98.26		99.71		102.7	
Overall % Mean Recovery	101.23							
Overall SD	2.564							
Overall % CV	3.04							

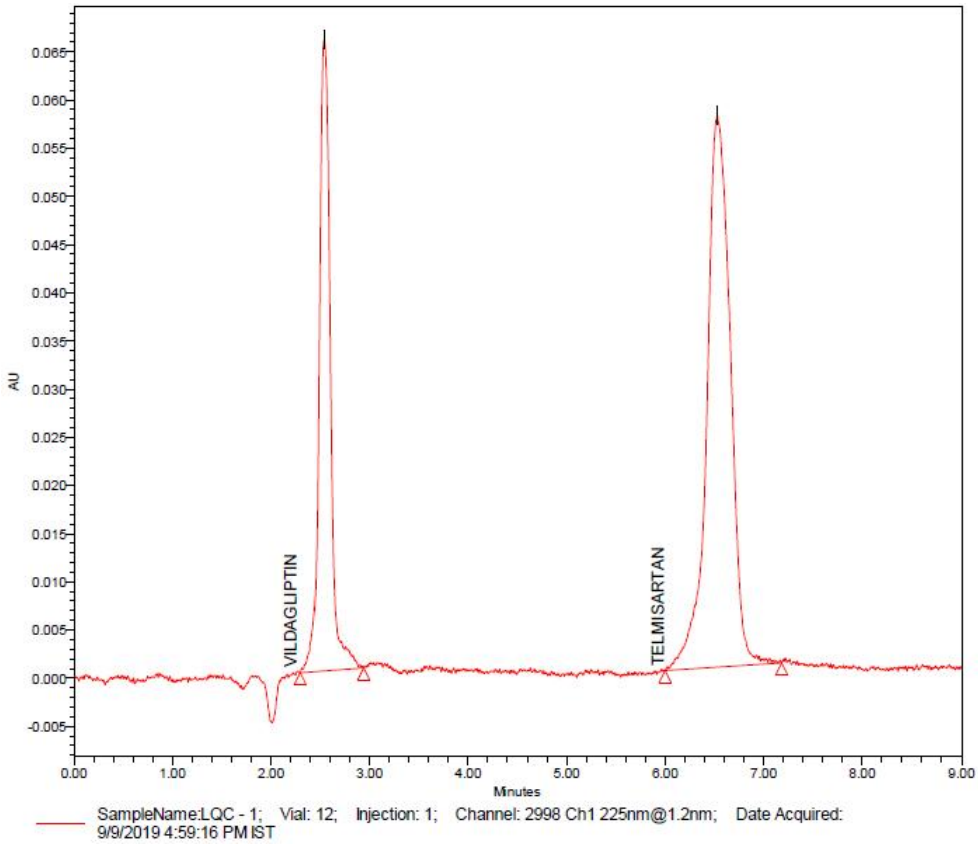

Fig. 5. Chromatograms of LQC at 2.810 µg/ml

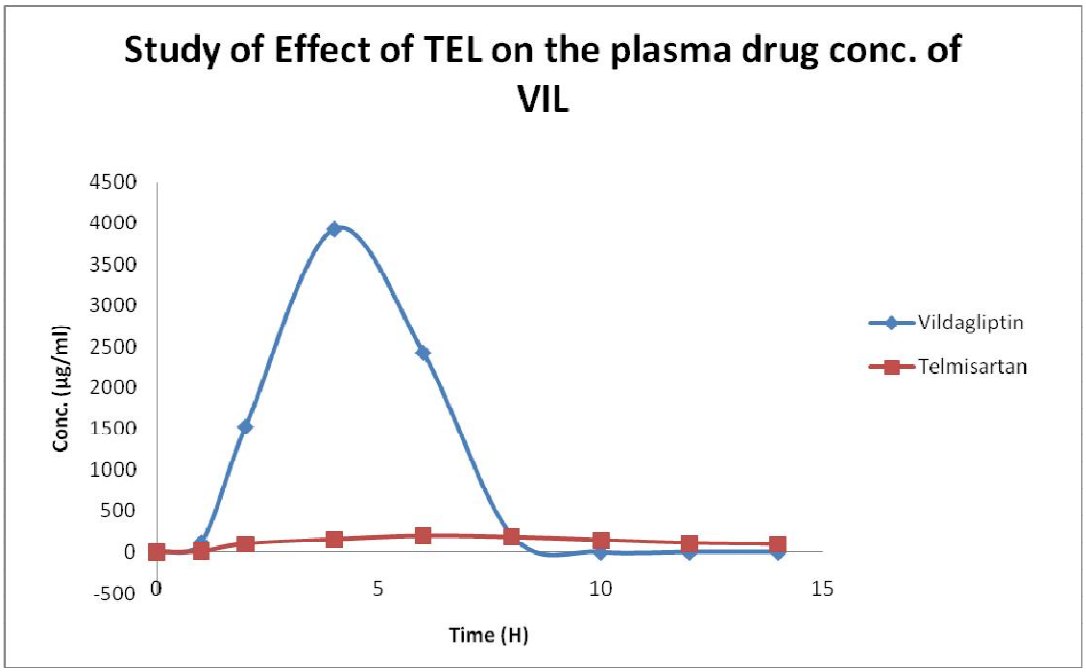

Fig. 6. Mean concentration versus time of Vildagliptin and Telmisartan

Table 5. Pharmacokinetic data of Vildagliptin and Telmisartan in rabbit plasma

Time (H)	Vildagliptin		Telmisartan	
	Peak areas	Concentration of the drug in plasma (µg/ml)	Peak areas	Concentration of the drug in plasma (µg/ml)
0	0	0	0	0
1	18703	123.65	2628	11.58
2	28172	1524.36	48900	101.36
4	37479	3921.36	65202	158.63
6	16806	2429.36	81629	201.48
8	6136	198.36	57853	185.36
10	0	BLQ	11419	148.25
12	0	BLQ	10515	110.63
14	0	BLQ	100833	99.25
C max (µg/ml)		921.36		201.48
T max (H)		4		6

4. CONCLUSION

The proposed HPLC-PDA method is rapid, sensitive and reproducible for the quantification of VIL and TEL in rabbit plasma with a wide linear dynamic range. It was validated and met all the requirements according to the USFDA standards guidelines with a high degree of accuracy and precision. Absence of matrix effects was adequately demonstrated. In addition, the stability study indicated that analytes were stable in plasma during the sample preparation process and other storage conditions. The lower LLOQ, smaller plasma volume and shorter run time make our new method particularly suitable and applicable to characterize the clinical pharmacokinetics and bioequivalence assay studies of VIL and TEL in rabbit plasma.

CONSENT

It is not applicable.

ETHICAL ARROVAL

The study was approved by Raghavendra Institute of Pharmaceutical Education and Research, Institutional Animal Ethics Committee (878/PO/RE/S/05/CPCSEA), Anantapur, Andhra Pradesh, India with the Approval no.: IAEC/XIII/05/RIPER/2019 Dt: 25.05.2019.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Singh SK, Manne N, Pal M. Synthesis of (S)-1-(2-chloroacetyl) pyrrolidine-2-carbonitrile: A key intermediate for dipeptidyl peptidase IV inhibitors. Beilstein J. Org. Chem. 2008;4:20-26.
2. Ramakrishna Nirogi, Vishwottam Kandikere, Koteshwara Mudigonda et. al., Sensitive liquid chromatography tandem mass spectrometry method for the quantification of sitagliptin, a DPP-4 inhibitor, in human plasma using liquid–liquid extraction. Biomedical Chromatography. 2008;22(2):214–222.
3. Mohammed Ishaq B, Vanitha Prakash K, Krishna Mohan G. Rp-HPLC method for simultaneous estimation of metformin and vildagliptin in bulk and its tablet formulation Journal of Global Trends in Pharmaceutical Sciences. 2012;3(3):747-754.
4. Ruilope LM. Telmisartan for the management of patients at high cardiovascular risk. Curr. Med. Res. Opin. 2011;27(8):1673-1682.
5. Moffat AC, Uselton MD, Widdop B. Clarke's analysis of drugs and poisons. 3rd ed. K. M. Varghese Company, Mumbai; 2004.

6. Nakatani M, Takeshi S, Ohki T, Toyoshima K. Solid telmisartan pharmaceutical formulations. US Patent. 0110813A1: 200.
7. Sweetman SC. Martindale - the complete drug reference. Pharmaceutical Press, London; 2005.
8. El-Bagary RI, Elkady EF, Ayoub BM. Spectrophotometric methods for the determination of sitagliptin and vildagliptin in bulk and dosage forms. Intern J Biomed Sci. 2011;7:55-61.
9. Pharne AB, Santhakumari B, Ghemud AS, Jain HK, Kulkarni MJ. Bioanalytical method development and validation of vildagliptin a novel dipeptidyl peptidase IV inhibitor by RP-HPLC method. Intern J Pharm Pharm Sci. 2012;4:119-123.
10. Barden AT, Salamon B, Schapoval EE, Steppe M. Stability indicating RP-LC method for the determination of vildagliptin and mass spectrometry detection for a main degradation product. J Chromatogr Sci. 2012;50:426-432.
11. Pushpalatha K, Ramachandran D. Development and validation for the simultaneous estimation of vildagliptin and metformin in tablet dosage form by RP-HPLC. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5(1):459-463.
12. Santhosha B, Ravindranath A, Sundari CH. Validated method for the simultaneous estimation of metformin hydrochloride and vildagliptin by RP-HPLC in bulk and the pharmaceutical dosage form. International Research Journal of Pharmaceutical and Applied Sciences. 2012;2(3):22-28.
13. Varma D, Lakshmanarao A, Dinda SL. Simultaneous determination of metformin and vildagliptin in solid dosage form by stability indicating RP-HPLC method, International Research Journal of Pharmacy. 2013;4(1):122-128.
14. Malakar A, Bokshi B, Nasrin D. Validation of RP-HPLC method for estimation of vildagliptin from tablet dosage form. International Journal of Pharmaceutical and Life Sciences. 2012;1(1):1-8.
15. Rane VP, Sangshetti JN, Shinde DB. Simultaneous high-performance liquid chromatographic determination of telmisartan and hydrochlorothiazide in pharmaceutical preparation. J. Chromatogr. Sci. 2008;46:887-891.
16. Lara FT, Maha FT, Mamoun IA, Manal HK, Adi IA. Simultaneous determination of irbesartan and hydrochlorothiazide in human plasma using HPLC coupled with tandem mass spectrometry: Application to bioequivalence studies. J. Pharm. Biomed. Anal. 2010;51:985- 990.
17. Vasu BR, Jaswanth KI, Nageswara RP, Vudagandla S, Venkateswarlu P. Simultaneous determination of telmisartan and amlodipine in human plasma by LC-MS/MS and its application in a human pharmacokinetic study. J. Pharm. Anal. 2012;2(5):319–326.
18. Christel H, Liane G, Ulrich K, Uwe K. Determination of telmisartan in human blood plasma Part II: Liquid chromatography-tandem mass spectrometry method development, comparison to immunoassay and pharmacokinetic study. Anal. Chimica. Acta. 2006;560:41-49.
19. Shah RP, Singh S. Identification and characterization of a photolytic degradation product of telmisartan using LC-MS/TOF, LC-MSn, LC-NMR and online H/D exchange mass studies. J. Pharm. Biomed. Anal. 2010;53:755-761.
20. Guidance for industry, bioanalytical method validation, us department of health and human services, food and drug administration Centre for Drug Evaluation and Research (CDER), Centre for Veternary Medicine (CVM) BP; 2001.
Available:http://www.fda.gov/cder/guidance/index.htm
Accessed on 02nd May 2019.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/64733