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Research Article

# OPTIMIZATION AND VALIDATION OF THE SIMULTANEOUS DETERMINATION OF VILDAGLIPTIN AND METFORMIN HCL IN HUMAN PLASMA BY RP-HPLC METHOD

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**ABSTRACT**

A new and simple method was developed for quantitative determination of Vildagliptin (VILD) and Metformin (MET) spiked with plasma using an Onyx $C_{18}$ Monolithic column connected with an Onyx $C_{18}$ guard cartridge and PDA detection at 220 nm. The mobile phase methanol (MeOH)/acetonitrile (ACN)/potassium dihydrogen orthophosphate ($KH_2PO_4$) was pumped at 0.4 mL/min. The method was linear between 0.5-2.5 µg/mL, statistically validated for its linearity, precision, and accuracy. The precision was found to be less than 1% of the assay method. It was found that the additives in the commercial tablet did not interfere with the method. The currently developed method can routinely use for the estimation of VILD and MET related compounds from the tablet dosage form spiked with plasma.

**Keywords:** Vildagliptin, Metformin, Plasma, RP-HPLC.

## INTRODUCTION

Vildagliptin (VILD) (**Fig.1**) is a potent dipeptidyl peptidase IV (DPP-IV) inhibitor, a drug for the treatment of type II diabetes[1]. DPP-IV inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type-2 diabetes. Chemically it is (S)-1-[N-(3-hydroxy-1- adamantyl) glycyl] pyrrolidine-2-carbonitrile.

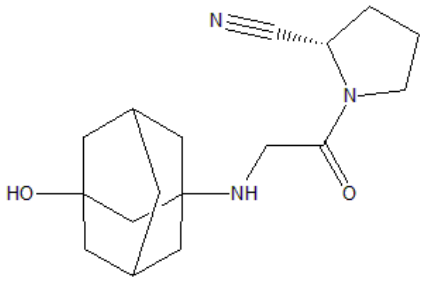


**Fig 1: Vildagliptin**

Metformin HCl (MET) (**Fig.2)**, chemically N, N-diethyl imidodicarbonimidic diamide hydrochloride. MET is a white powder. MET is freely soluble in water, slightly soluble in ethanol (95%), practically insoluble in acetone, ether, and chloroform. Literature review reveals that some analytical procedures have been accounted for estimation of VILD and MET individually as stability indicating and in biological fluids or in combination with different drugs in pharmaceutical dosage forms.

As of late HPLC[2,3], UV-Spectroscopy[4], LC-MS[5], spiked with plasma[6] and Quality by design technique[7] have been accounted for the simultaneous determination of VILD and MET in pharmaceutical dosage forms and biological fluids which are either monotonous or costly techniques.

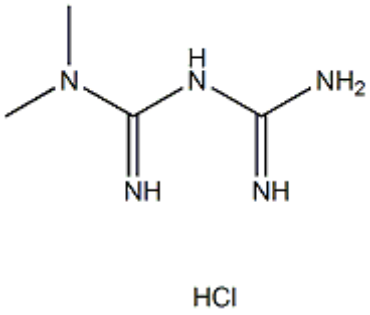


**Fig 2: Metformin HCL**

## METHODS

Chromatographic measurements were made on an RP-HPLC Shimadzu (Tokyo, Japan) model which consisted of an LC-20AD solvent delivery module, SPD-M20A prominence diode array detector, a Rheodyne injector (model 7125, USA) valve fitted with a 20µl loop. The system was controlled through a system controller (SCL-10A) and a personal computer using a Shimadzu chromatographic software (LC Solution, Release 1-11SP1) installed on it. The mobile phase was degassed using Branson sonicator (Branson Ultrasonic Corporation, USA). Absorbance spectra were recorded using an UV-double beam spectrophotometer (Systronices 2202 Model UV-1601PC, Japan) employing a quartz cell of 1 cm of path length.

### Chemicals and reagents

Working standards of VILD and MET were purchased from Biotech Solutions, New Delhi. MeOH, ACN of HPLC grade and $KH_2PO_4$ was of analytical reagent grade supplied by M/S SD Fine Chemicals, Mumbai, India. The HPLC grade water was prepared by using Milli-Q Academic, Millipore, and Bangalore, India. The tablet dosage form GALVUSMET (Batch No' 17JX009A, MFG:

NOV 2016, EXP: DEC 2018) was purchased from Medicine Chamber, Park Town, Chennai, India. Plasma sample has been collected from the Blood bank of RMMC&H, Annamalai University, Chidambaram, as gift sample for this research work.

**Chromatographic condition**

Chromatographic separation was carried out on an Onyx $C_{18}$ monolithic column (100mm× i.d., 5µm) connected with an Onyx $C_{18}$ guard cartridge (4mm×3mm i.d., 5µm). The mobile phase consisted of MeOH: ACN: $KH_2PO_4$ (pH 4 ±0.5) (33.9:10:56.1). The method was optimized by using the D-optimal design from Design of Expert software (Trial of version 11 of Design-Expert® Software 2017). A wavelength of 220 nm was selected for detection. The injection volume of the sample was 20µl. The HPLC system was used in an air-conditioned laboratory atmosphere (20±2°c).

**Preparation of Standard Solutions**

A stock standard solution of VILD and MET (1mg/ml) was prepared in the mobile phase. The readied stock arrangements were put away at 4°C ± 0.05, subsequently shielded from light. Working standard solutions were freshly arranged by diluting the stock solutions with mobile phase during analysis day. Calibration curves revealing peak area ratios of VILD and MET were built up in the range of 0.5-2.5µg/ml.

**Selection of detection wavelength**

VILD and MET showed significant absorbance at 220 nm using PDA detector.

**Preparation of Sample Solution**

Serial dilutions of analyte were prepared in the mobile phase and 1ml of each dilution was spiked into 100µL of plasma in a polypropylene centrifuge tubes. Then all the tubes were centrifuged for 20 min at 3000 rpm. Supernatant was collected in another Eppendorf tube and 20µL supernatant was injected into the analytical column.

**Assay validation**

As per the guidelines of the [ICH] Q2 [R1], the developed RP-HPLC method was validated[8].

**Linearity and range**

For linearity, the standard solution of VILD and MET ware applied in the concentration of 0.5-2.5 µg/mL. The graph of peak area versus concentration was plotted. Least square linear regression analysis was done as well as the correlation coefficient, intercept, and slope was calculated[9].

**Sensitivity**

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated using formula 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the linearity plot[10].

**Specificity**

The specificity of the method was calculated by comparing test results obtained from the analysis of sample solution with standard drug[11].

**Precision studies**

Precision was calculated by taking 1, 1.5, 2 µg/mL concentration of VILD and MET sample ware analyzed six times on a similar day to find out any differences in the results[12].

**Accuracy studies**

Accuracy is the closeness in the agreement between the accepted true value or a reference value and the actual results obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the mixture of the sample further analyzed. For accuracy studies, three different concentration of solution such as 0.8 µg/mL, 1 µg/mL, and 1.2 µg/mL was used. For these, each concentration was injected and the mean % recovery was calculated[13].

**RESULT AND DISCUSSION**

**Chromatographic development**

Chromatographic analysis was developed using an Onyx $C_{18}$ Monolithic column (100mm× i.d., 5µm) connected with an Onyx $C_{18}$ guard cartridge (4mm×3mm i.d., 5µm). The mobile phase consists of MeOH: ACN: $KH_2PO_4$ (pH 4 ±0.5) (33.9:10:56.1) and that was supplied at a flow rate of 0.4 mL/min. The method was optimized by using Design of Expert software. A wavelength of 220 nm was selected for detection. Fig. 3 shows the optimized chromatogram of VILD and MET.

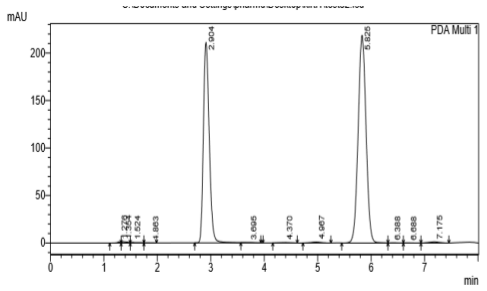


**Fig. 3: Optimized chromatogram of VILD and MET**

**Validation of the method**

**Linearity**

The analyte response was linear ($r^2$=0.999) over the concentration range of 0.5–2.5 µg/mL of VILD and MET. The results were shown in Table 1. The calibration curve was shown in Fig. 4 and Fig. 5. The curve shows the selected concentration gives acceptable accuracy and precision over a wide concentration range. The results demonstrate that an excellent correlation coefficient between the absorbance and concentration of VILD and MET.

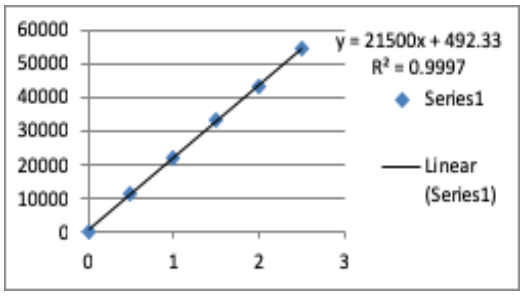


**Fig. 4: Linearity graph of VILD**

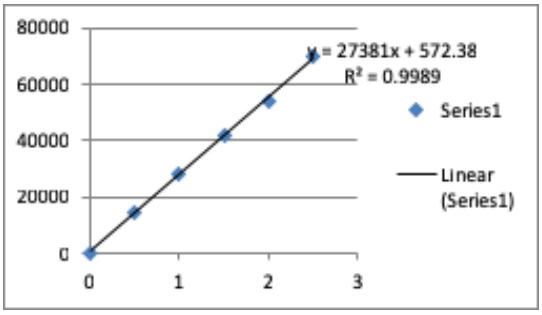


**Fig. 5: Linearity graph of MET**

**Table 1: Linear regression analysis of VILD and MET**

| Parameters | VILD | MET |
|---|---|---|
| Linearity range (µg/mL) | 0.5-2.5 | 0.5-2.5 |
| Correlation Coefficient ($r^2$) | 0.999 | 0.998 |
| Slope | 21500 | 27381 |
| Intercept | 492.3 | 572.3 |

**Sensitivity**

The LOD was found to be 0.00308 and 0.0.003088 µg for VILD and MET respectively. The LOQ for VILD and MET were found to be 0.00935 and 0.009359 µg respectively representing good sensitivity of the method.

**Specificity**

The chromatograms obtained from standard and sample solutions are not interfering, the method is highly selective.

**Precision**

In the estimation of VILD and MET (Table 2) showed that the % RSD was <2% during the analysis. These low values of RSD show that the precision of the method is good.

**Table 2: Precision studies of VILD and MET**

| Drug | Actual Concentration | Precision Data | % RSD |
|---|---|---|---|
| VILD | 1.5 µg | 99.99 | 0.0109 |
| MET | 1.5 µg | 99.99 | 0.0072 |

**Accuracy**

The study of accuracy reveals influences of additives that are usually present in the dosage forms on the quantitative parameters. The recovery study data presented in Table 3 indicates that the accuracy of the quantification of VILD and MET ware more than 99%, which indicate that the proposed simultaneous RP-HPLC method is reliable for the estimation of marketed formulation used in the study.

**Table 3: Results of recovery studies of VILD and MET**

| Amount taken | | Amount added | | % recovery | | %RSD | |
|---|---|---|---|---|---|---|---|
| VILD | MET | VILD | MET | VILD | MET | VILD | MET |
| 1 | 1 | 0.8 | 0.8 | 99.2 | 98.8 | 0.0078 | 0.0072 |
| 1 | 1 | 1 | 1 | 100 | 99 | 0.007 | 0.0058 |
| 1 | 1 | 1.2 | 1.2 | 99.18 | 99 | 0.0067 | 0.005 |

**ANALYSIS OF A MARKETED PREPARATION WITH SPIKED PLASMA**

The results obtained for the amount of VILD and MET in tablet powder spiked with human plasma as against the label claims were in good-great assertion signifying that there is no interference from any of the excipients presents in the tablet. The percent assay was found to be 99.2% and 99.4%, for VILD and MET respectively.

**CONCLUSION**

The purpose of this investigation was to develop and validate a method using a simple, price effective, sensitive, precise, and accurate RP-HPLC for the routine determination of VILD and MET in bulk, plasma, and pharmaceutical preparations. The proposed method is suitable for pharmaceutical analysis in various analytical laboratories. As retention time and run time were very short it requires less mobile phase, making it more economical and rapid. Hence the method can be used for the analysis of a large number of samples.

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