

# Application of Validated HPLC Method for Degradation Study of Vildagliptin and Metformin HCl

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## Abstract

A novel and simple reverse phase liquid chromatographic method has been established for the determination of Vildagliptin and Metformin HCl and studies its degradation pattern in pharmaceutical dosage forms. Vildagliptin and Metformin HCl is used to control Type 2 Diabetes. The proposed work was performed on Younglin (S.K) isocratic System UV Detector C18 column (150 mm × 4.6 mm). A mixture of Potassium Phosphate, mobile phase in this method with flow rate 0.7 ml/min (UV detection at 203 nm) and the method was validated as per ICH guidelines. Forced degradation studies were performed by exposing the drug Vildagliptin and Metformin HCl to acidic, alkaline, oxidation and thermal stress degradations. The proposed RP-HPLC method was found to be robust and specific and this method is suitable for the assay of pharmaceutical dosage forms as well as kinetic studies.

**Keywords:** Vildagliptin, Metformin HCl, RP-HPLC, validation, stability-indicating.

## 1. Introduction

Vildagliptin is (S)-1-[N-(3-hydroxy-1 adamanty) glycy] pyrrolidine-2-carbonitrile. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP-4, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas.[1-3]

## 2. Experimental

### 2.1 Chemicals and reagents

Vildagliptin and Metformin HCl were provided from Merck Laboratories Ltd. HPLC grade Potassium Phosphate buffer pH – 3.2 with Orthophosphoric acid, acetonitrile, sodium hydroxide were procured from Merck Ltd. High pure water was prepared by using Milipore Mili Q plus purification system.

### 2.2 HPLC instrumentation and conditions

A High performance liquid chromatograph system, with LC solutions data handling system with isocratic system. The data was recorded using Autocro-3000 solutions software. The sample separation was performed

on a Shimadzu Primesil C18 (4.6x 150 mm) with the mobile phase consisting of Acetonitrile and Potassium Phosphate buffer pH-3.2 with a ratio of 40:60 (v/v) at ambient temperature. The flow rate was kept at 0.7 ml/min and the determination wavelength was 225 nm.

### 2.2.1 Mobile Phase

Mix 700 ml of Acetonitrile to the buffer, the mobile phase was sonicated for 15 min and then it was filtered through 0.45 µm membrane filter paper.

### 2.2.2 Standard Solution

The standard was dissolved with mobile phase to 5 mg/ml. The test samples were dissolved with mobile phase. With the optimized chromatographic conditions, a steady baseline was recorded, the standard solution was injected and the chromatogram was recorded. The procedure was repeated for the sample solution.

### 2.3 Forced degradation studies

Vildagliptin and Metformin HCl was allowed to hydrolyze in different strengths of base and acid (0.1 N) and hydrogen peroxide (0.1 N). The combination was studied for its neutral degradation. Further it is important to

note that from the chromatograms (Figure 2 and 3), it is evident that although the degraded peaks are observed. The combination Vildagliptin and Metformin HCl are stable under the applied stress conditions like acid, base, oxidative, neutral degradation states.

#### 2.4 Linearity

The calibration curve showed good linearity in the range of 10-50 mg/ml for Metformin HCl and 1-5 mg/ml For Vildagliptin HCl. The combination with RSD- 0.95 (Figure). A typical calibration curve has the regression equation of  $Y=103.0X+123$   $R^2 = 0.999$  for Metformin HCl and  $Y=85.97X-3.638$   $R^2 = 0.999$  for Vildagliptin.

#### 2.5 Precision

The results of system precision (% RSD = 0.97) for Metformin HCl and Vildagliptin method precision are found within the prescribed limit of ICH guidelines.

#### 2.6 Intra-assay and Inter-assay

The intra and inter-day variation of the method was carried out and high values of mean assay and low values of standard deviation and % RSD within a day and day to day variations for Vildagliptin and Metformin HCl revealed that the proposed method is precise in (Table 2)

#### 2.7 Method robustness

Influence of small changes in chromatographic conditions such as change in flow rate (10%), organic content in mobile phase (2%), wavelength of detection

(5%) and pH of buffer in mobile phase (0.2%) studied to determine the robustness of the method are also in favour of the developed RP-HPLC.

#### 2.8 LOD and LOQ

The minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) were found to be for Metformin HCl- 0.3 and 0.93 and for Vildagliptin HCl- 0.11 and 0.34 respectively.

### 3. Results and Discussion

The present study is the report on stability indicating assay of combination Vildagliptin and Metformin HCl in presence of degradation products by HPLC. In this method isocratic elution method was selected for analysis of combination. Because, it gave better base line separation and peak width, which is suitable for routine analysis of combination. The developed method was validated as per ICH guidelines.

Stability testing forms an important part of process of drug product development. The purpose of stability testing is to provide evidence on how the drug quality substance varies with time under influence of various environmental factors such as temperature, humidity, and light, and enables recommendations of storage conditions, retest periods and shelf life to be established.

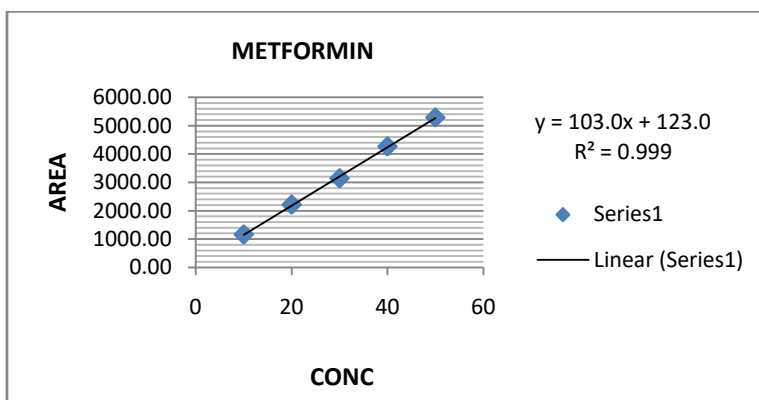


Figure 1A: Linearity Curve for Metformin HCl

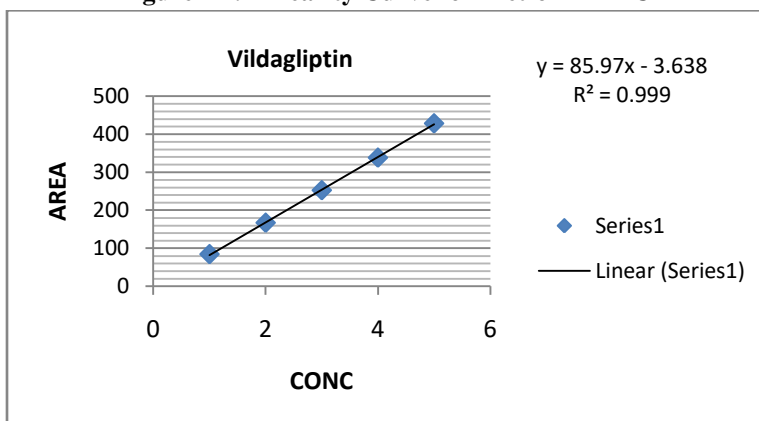


Figure 1B: Linearity Curve for Vildagliptin

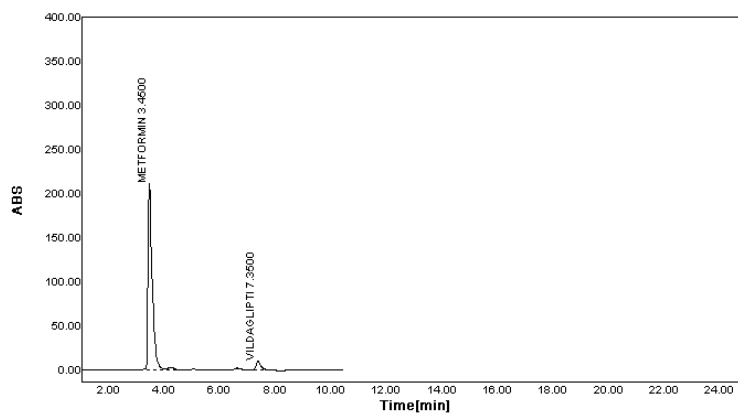
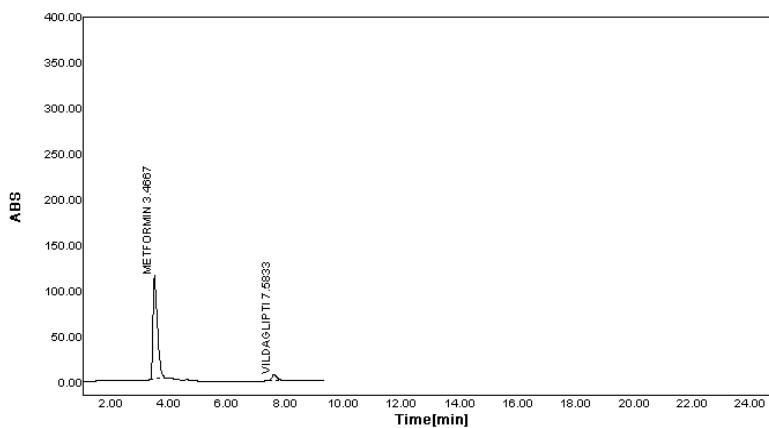
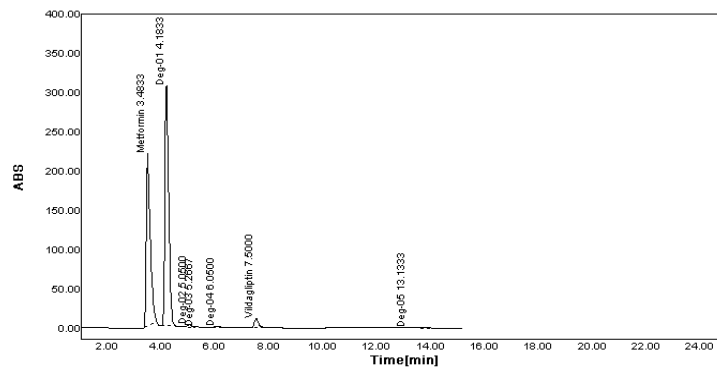
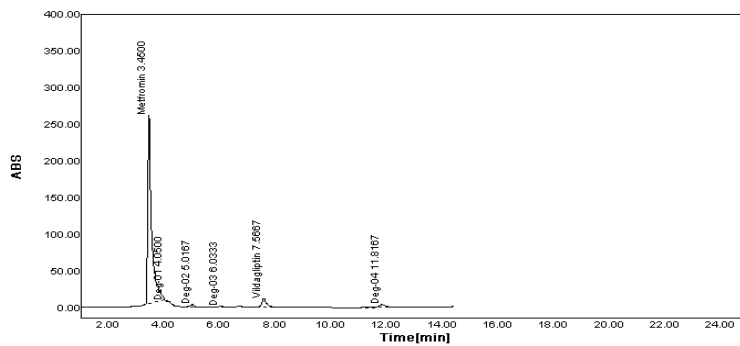


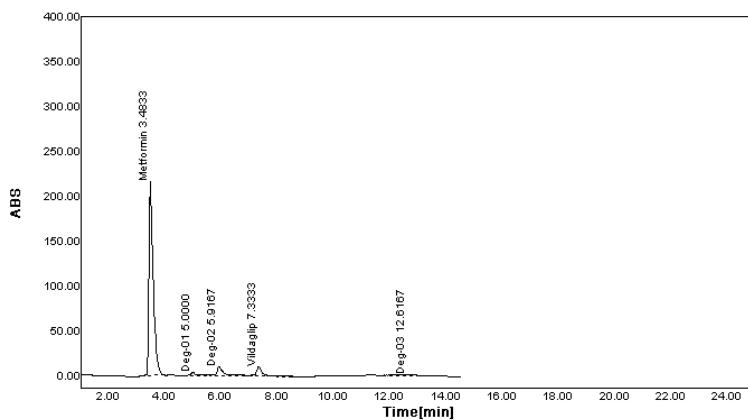
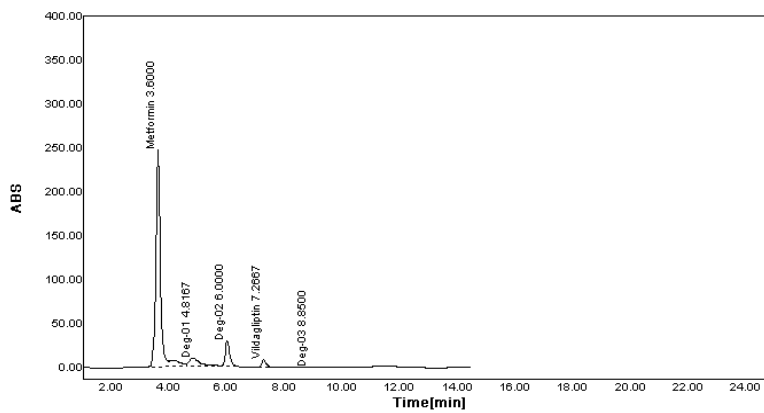
Figure 2: Chromatogram of Vildagliptin and Metformin HCl



(a)



(b)



(c)

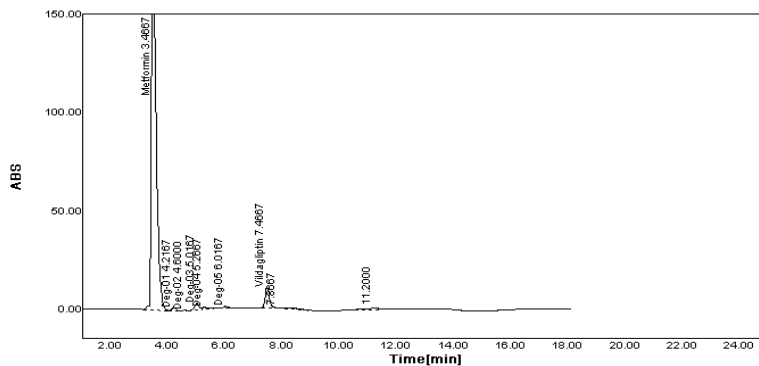
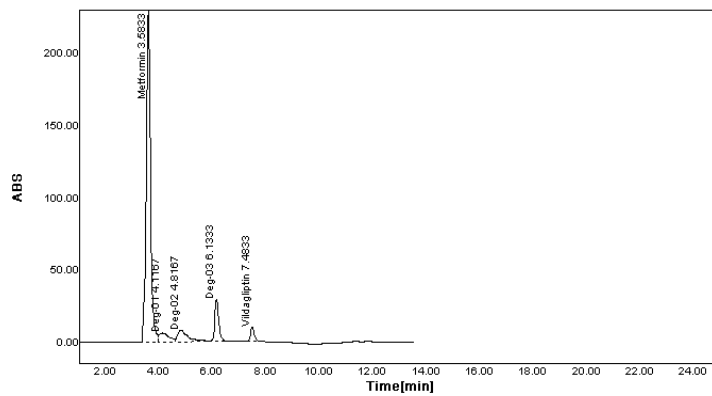


Figure 3: Chromatograms of (a) Oxidative degraded sample (b) Acid Hydrolysed degraded (c,d,) Alkali degradation (e,f) Neutral degradation

**Table 1: Results of force degradation studies of Vildagliptin and Metformin HCl**

Stress Condition/duration/solution	Degradation (%)
Acid Degradation (0.1 N) after 1hr	11.61
Acid Degradation (0.1 N) after 2hr	11.83
Alkaline Degradation (0.1 N) after 1hr	6.13
Oxidative Degradation (0.1 N) after 1hr	64.84
Neutral Degradation (0.1 N) after 1hr	12.61
Neutral Degradation (0.1 N) after 2hr	11.20

**Table 2: Inter-Assay Precision data of proposed RP-HPLC method**

	Mean (%w/w)	SD	% RSD
Assay-1	99.06	0.11	0.63
Assay-2	101.00	1.37	1.37
Intra Assay	100.91	3.23	0.08

**Table 3: Intra-Assay Precision data of proposed RP-HPLC method**

	Mean (%w/w)	SD	% RSD
Assay-1	101.18	6.97	0.32
Assay-2	97.59	11.42	0.36
Inter Assay	100.91	3.23	0.08

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