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Application of Validated HPLC Method for Degradation Study of Sitagliptin and Metformin HCl

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Abstract

A novel and simple reverse phase liquid chromatographic method has been established for the determination of Sitagliptin and Metformin HCl and studies its degradation pattern in pharmaceutical dosage forms. Sitagliptin and Metformin HCl is used to control Type 2 Diabetes. The proposed work was performed on Younglin (S.K) isocratic System UV DetectorC18 column (150 mm × 4.6 mm). A mixture of Potassium Phosphate buffer pH-3.2 with orthophosphoric acid and acetonitrile was used as 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 Sitagliptin 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: Sitagliptin, Metformin HCl, RP-HPLC, validation, stability-indicating.

1. Introduction

Sitagliptin R)-4-oxo-4-[3-(trifluoromethyl) 5,6dihy dro [1,2] triazolo[4,3-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine. Sitagliptin[1-4] is a DPP-4 inhibitor, which is believed to exert its actions in patients with type 2 diabetes by slowing the inactivation of incretin hormones. Concentrations of the active intact hormones are increased by sitagliptin, thereby increasing and prolonging the action of these hormones. Incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP), are released by the intestine throughout the day, and levels are increased in response to a meal. These hormones are rapidly inactivated by the enzyme, DPP-4. Sitagliptin demonstrates selectivity for DPP-4 and does not inhibit DPP-8 or DPP-9 activity in vitro at concentrations approximating those from therapeutic doses.N,Ndimethylimidodicarbonimidicdiamide Metformin[5-21] improves hyperglycemia primarily through its suppression of hepatic glucose production (hepatic gluconeogenesis).Metformin activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats; activation of AMPK is required for Metformin's inhibitory effect on the production of glucose by liver cells.

2. Experimental Section

2.1 Chemicals and reagents

Sitagliptin and Metformin HCl were provided from Merck Laborateries 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 MiliporeMili 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 Acetonitirle and Potassium Phosphate buffer pH-3.2 with a ratio of 35:65 (v/v) at ambient temperature. The flow rate was kept at 0.7 ml/min and the determination wavelength was 215 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

Sitagliptin 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 3), it is evident that although the degraded peaks are observed. The combination Sitagliptin 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 20-60 mg/ml for Metformin HCl and 1-3 mg/ml For Sitagliptin HCl. The combination with RSD- 0.95 (Figure 1).A typical calibration curve has the regression equation of y =106.1x+486.7 R^2= 0.999 for Metformin HCl and y = 164.0x-65.22R^2= 0.999 for Sitagliptin.

2.5 Precision

The results of system precision (% RSD = 0.97) for Metformin HCl and Sitagliptin, 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 Sitagliptin and Metformin HCl revealed that the proposed method is precise in (Table2)

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 favor 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- 1.0176 and 3.083 and for Sitagliptin HCl- 0.050 and 0.3364 respectively.

2.9 Specificity and stability in analytical solution

The results of specificity indicated that the peak was pure in presence of degraded sample. It is important to mention here that the combination Sitagliptin and Metformin HCl was stable in solution from up to 24 hrs at 25°C.

The results of linearity, precision, inter and intra-assays, method robustness, LOD, LOQ and specificity and stability in analytical solution established the validation of the developed RP-HPLC assay for analysis of Sitagliptin and Metformin HCl.

3. Results and Discussion

The present study is the report on stability indicating assay of combination Sitagliptin 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, light,and enables recommendations of storage conditions, retest periods and shelf life to be established.

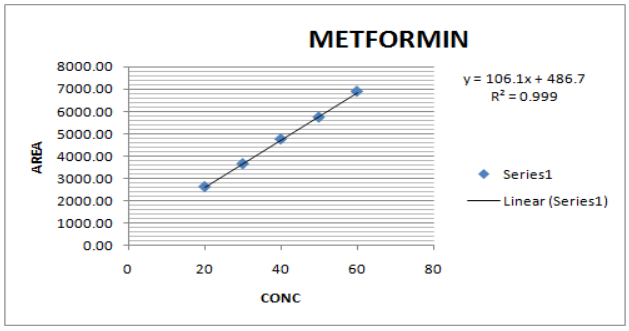


Figure 1A: Linearity Curve for Metformin HCl

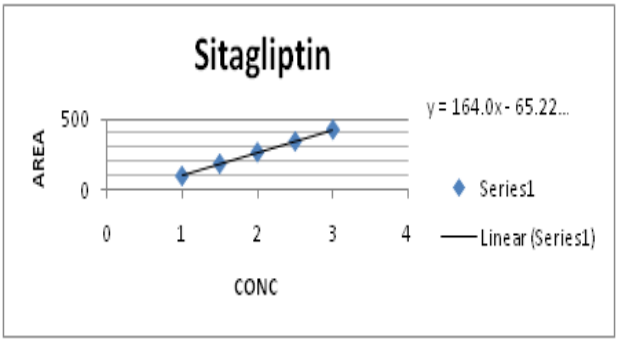


Figure 1B: Linearity Curve for Sitagliptin

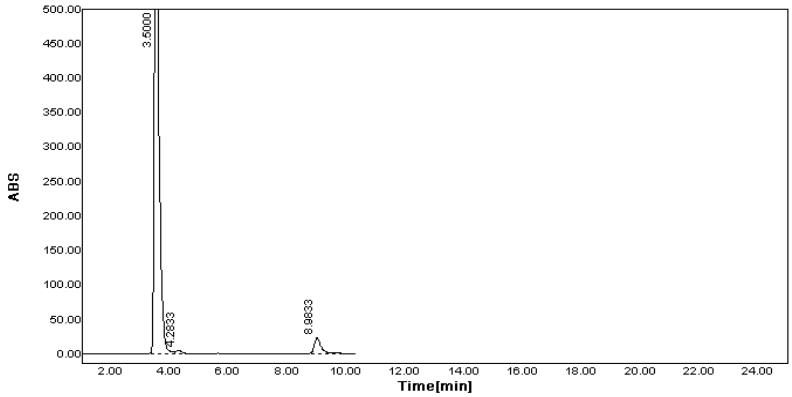
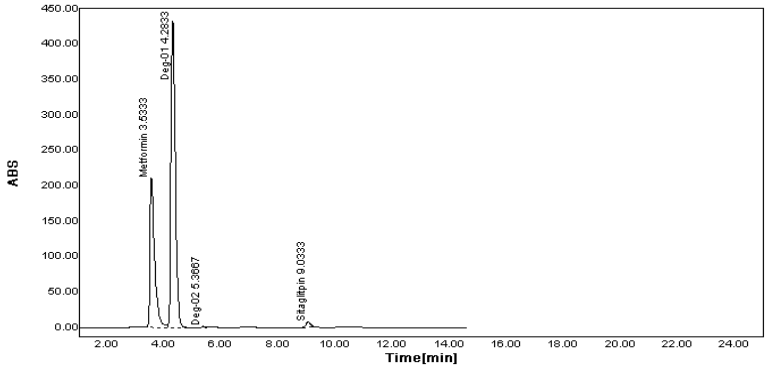
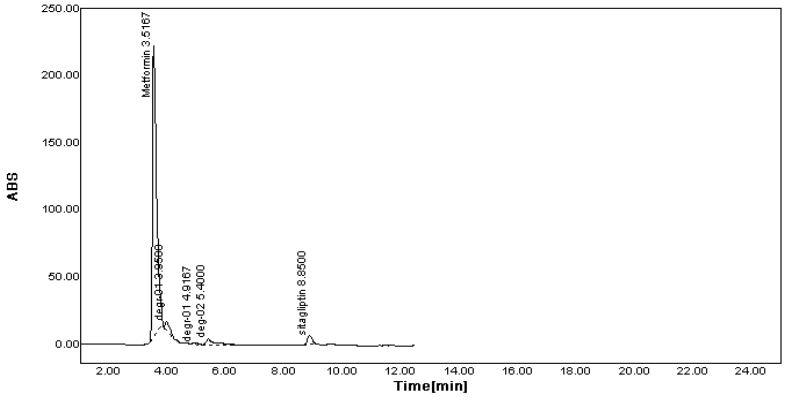


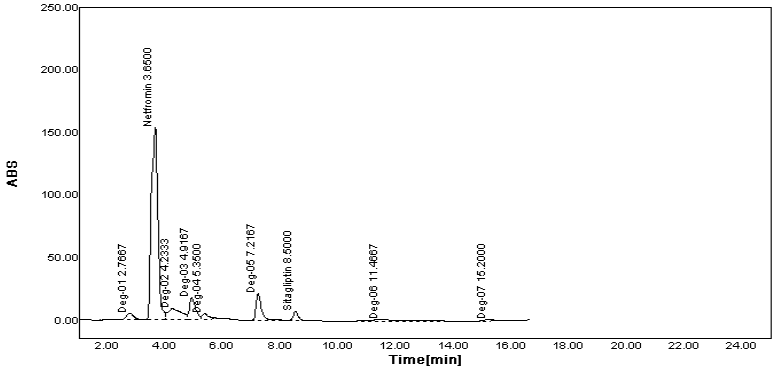
Figure 2: Chromatogram of Sitagliptin and Metformin HCl



(a)



(b)



(c)

Figure 3: Chromatograms of (a) Oxidative degraded sample (b) Acid Hydrolysed degraded (c) Base Hydrolysed degraded sample

Table 1: Results of force degradation studies of Sitagliptin and Metformin HCl

Stress Condition/duration/solution	Degradation (%)
Acid Degradation (0.1 N) after 1hr	0.60
Acid Degradation (0.1 N) after 2hr	1.69
Alkaline Degradation (0.1 N) after 1hr	7.72
Oxidative Degradation (0.1 N) after 1hr	64.84
Neutral Degradation (0.1 N) after 1hr	1.51
Neutral Degradation (0.1 N) after 2hr	1.38

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

	Mean (%w/w)	SD	%RSD
Assay-1	102.62	1.79	0.97
Assay-2	98.45	4.04	1.59
Intra Assay	102.97	25.67	5.71

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

	Mean (%w/w)	SD	%RSD
Assay-1	102.52	1.79	0.79
Assay-2	98.45	4.01	1.56
Inter Assay	102.97	24.67	5.59

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