

# Development and Validation of a Method for Simultaneous Determination of Metformin Hydrochloride and Sitagliptin Phosphate in a Formulation by RP-HPLC

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## Abstract

Present study was aimed to develop and validate a reverse-phase high-performance liquid chromatography method for simultaneous determination of sitagliptin phosphate and metformin hydrochloride in a marketed formulation. The drug separation was performed on Hibar-240, Lichrosphere-100 C18 ODS (250 × 4.6 mm, 5 μm) column, at a flow rate of 1 mL/min. The mobile phase used was a mixture of methanol: potassium di-hydrogen phosphate buffer at a ratio of 70:30 v/v. The detection was carried out at a wavelength of 266 nm. The retention times of sitagliptin phosphate and metformin hydrochloride were found as 6.1 and 4.9 min respectively. Linear calibration curves with good correlation coefficients were obtained over the concentration ranges of 10 - 50 μg/mL for sitagliptin and 20 - 100 μg/mL for metformin. The limit of detection was 0.016 and 0.14 μg/mL and the limit of quantification was 0.048 and 0.42 μg/mL for sitagliptin phosphate and metformin hydrochloride respectively. Validation of the method demonstrated system selectivity, specificity, linearity, accuracy and precision. The developed method was found useful in the simultaneous analysis of sitagliptin phosphate and metformin hydrochloride in formulation.

## Keywords

Sitagliptin Phosphate, Metformin Hydrochloride, Reverse Phase High Performance Liquid Chromatography (RPHPLC), International Committee on Harmonization (ICH) Guidelines, Limit of Detection (LOD), Limit of Quantitation (LOQ), Linearity, Precision, Accuracy & Reproducibility

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## 1. Introduction

Dipeptidyl peptidase-4 (DPP-4) inhibitors are emerged as a new class of oral hypoglycemic agents in the treatment of diabetes. This class of agents is acted by the inhibition of the incretins inactivation, specifically glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycemic control. Sitagliptin phosphate is an orally active DPP-4 inhibitor and it is chemically 7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-[3-(trifluoromethyl)-1,2,4-triazolo[4,3- $\alpha$ ]pyrazine phosphate (1:1) monohydrate. Sitagliptin also enhances glycemic control and shows a positive influence on the growth of  $\beta$ -cells in pancreatic islets [1]-[4]. Metformin (MET), N,N-dimethylimidodicarbonimidic diamide is a biguanide drug that stimulates glycolysis in peripheral tissues [5]. Metformin lowers hepatic glucose production via a still unknown mechanism [6]. Insufficient glycemic control with first line drugs results into combination therapy with either oral hypoglycemic agents or insulin to patient's treatment regimen [7].

Recently, the combination of sitagliptin and metformin has been recommended for use in the treatment of diabetes mellitus to improve glycemic control [8]. Already few methods were reported for the determination of sitagliptin in pharmaceutical formulations or biological samples by spectrophotometry and HPLC [5]. Analytical methods for determination of metformin include normal phase chromatography (silica and cyano), cation exchange chromatography, ion pair chromatography and reversed phase chromatography with UV or mass spectrometric detection [9].

The present work presents development and validation of a new and simple RP-HPLC method for simultaneous determination of metformin hydrochloride and sitagliptin phosphate in a formulation.

## 2. Experimental

### 2.1. Materials and Reagents

HPLC grade acetonitrile and methanol were purchased from Sigma Aldrich (Bangalore, India). Analytical grade solvents namely Butanol, Methanol, Chloroform, Diethyl amine, Potassium dihydrogen phosphate and Formic acid were purchased from Sigma Aldrich (Bangalore, India). Pharmaceutical grade sitagliptin was a gift sample from Merck Sharp & Dohme (Cairo, Egypt). Metformin was generous gift sample obtained from Aurobindo Pharmaceuticals Pvt Ltd. (Hyderabad, India). Janumet<sup>®</sup> tablets nominally containing 50 mg of sitagliptin phosphate monohydrate and 500 mg of metformin per tablet were all kindly supplied by Merck Sharp and Dohme Co. (Cairo, Egypt).

### 2.2. Instrumentation and Chromatography

The HPLC system consisted of a Shimadzu LC-20AD pumps, SPD-20A UV detector, SPD-M10A VP diode array detector. The method was developed on Hibar-240, Lichrosphere-100 C18 ODS (250  $\times$  4.6 mm, 5  $\mu$ m) column. The data acquisition was performed by processing software "LC Solution" (Shimadzu Corp.).

The mobile phase was a mixture of methanol and potassium di-hydrogen phosphate buffer taken at a ratio of 70:30 v/v, delivered at a flow rate of 1 mL/min. The column was maintained at 30°C and the detection was carried out at a wavelength of 261 nm (PDA, UV). The injection volume was 20  $\mu$ L.

### 2.3. Preparation of the Standard Solutions

#### 2.3.1. Stock and Working Standard Solutions

The stock solution of sitagliptin (5000  $\mu$ g/mL) and metformin (50,000  $\mu$ g/mL) was prepared by taking 50 mg and 500 mg of sitagliptin and metformin respectively into a 10 mL volumetric flask. Standard drugs were dissolved in 3 mL methanols and sonicated for 5 minutes followed by made up to the volume with the addition of methanol. From the stock solutions, working solutions of sitagliptin and metformin were prepared in 1 - 1000  $\mu$ g/mL and 10 - 10,000  $\mu$ g/mL concentration range, respectively.

#### 2.3.2. Sample Preparation

Ten tablets were weighed and the coats were removed. An accurately weighed amount of finely powdered janumet tablets equivalent to 500 mg of metformin and 50 mg of sitagliptin were taken into a volumetric flask and 3 mL of methanol was added. Followed by sonication for 25 minutes and then made up to 10 mL with methanol.

The solutions were filtered and prepared dilutions to 1 µg/mL of sitagliptin and 10 µg/mL of metformin.

### 3. Results and Discussion

#### 3.1. Method Validation

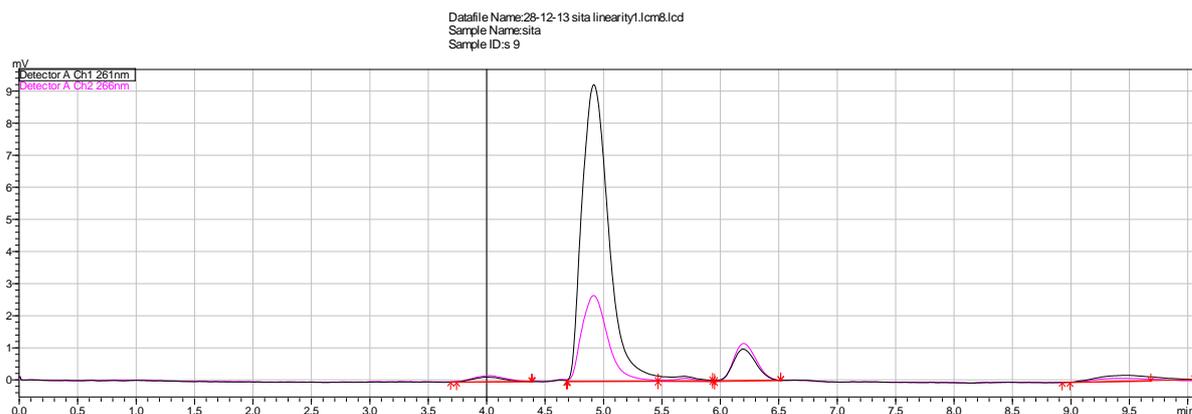
The method validation was performed in accordance with ICH guidelines [10]. The parameters assessed were linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, reproducibility and robustness.

##### 3.1.1. Selectivity and Specificity

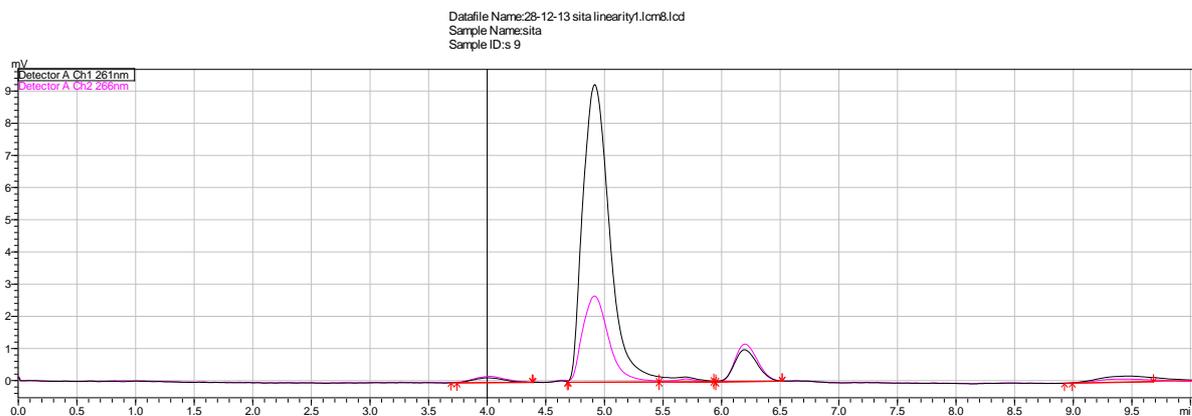
A representative chromatogram of the standard drugs is shown in **Figure 1**. No additional peaks were observed. **Figure 2** shows the chromatograms of standard drug contain 98.15% and 99.4% of sitagliptin and metformin respectively. The retention time of metformin and sitagliptin were found at 4.9 and 6.1 min respectively. Assay (%) 98 - 102% this is in accordance with ICH guidelines. Hence the developed method can be routinely employed for the simultaneous estimation of sitagliptin and metformin in the marketed formulations. Assay values of sitagliptin and metformin were presented in **Table 1**.

##### 3.1.2. Linearity

Linear calibration curves with correlation coefficients greater than 0.9999 were obtained over a concentration range of 10 - 50 µg/mL for sitagliptin and 20 - 100 µg/mL for metformin. The typical equation of the calibration curves of the both drugs were obtained with r value 0.998. The results shown that within the concentration range indicated there was an excellent correlation between peak area ratio and each concentration of the sitagliptin and metformin.



**Figure 1.** Chromatogram of standard.



**Figure 2.** Chromatogram of sample.

### 3.1.3. Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated for sitagliptin and metformin according to ICH guidelines by taking standard deviation and slope from the calibration curve. LOD and LOQ were calculated by using below formulae

$$\text{LOD} = 3.3 \times \text{SD} / \text{slope}; \text{LOQ} = 10 \times \text{SD} / \text{slope}$$

LOD and LOQ values were presented in **Table 2**.

### 3.1.4. Precision

Precision of the analytical method was determined by replicating the process in triplicates at 3 different concentrations of sitagliptin and metformin (combination) and chromatograms were recorded. Precision was calculated as a percentage relative standard deviation. The relative standard deviation should be less than 2 according to ICH guidelines. The % relative standard deviation of sitagliptin (2, 4, 6 µg/mL) and metformin (20, 40, 60 µg/mL) was found less than 2%. Precision data of sitagliptin and metformin were given in **Table 3** and **Table 4** respectively.

### 3.1.5. Accuracy and Recovery Studies

To the pre analyzed samples known amount of standard solutions in different concentration levels (50%, 100% and 150%) were added. For these solutions chromatograms were developed to record accuracy and recovery. The recoveries of sitagliptin and metformin were determined at 3 concentration levels (low, medium, high). Accuracy of the method was treated as percent recovery. Recoveries of sitagliptin (**Table 5**) and metformin (**Table 6**) were in between 98% - 102%. This is in accordance with ICH guidelines. Therefore method was found to be accurate.

**Table 1.** Assay of sitagliptin and metformin.

Parameters	Sitagliptin	Metformin
Standard peak area	15,067	148,722
Test peak area	14,985	146,128
Average weight	704 mg	704 mg
Label claim	50 mg	500 mg
% Purity of standard	99.8	99.9
Amt obtained	49.25 mg	490.0
% Assay	98.15%	99.4%

**Table 2.** LOD & LOQ.

	Sitagliptin	Metformin
LOD	0.016 µg/mL	0.14 µg/mL
LOQ	0.048 µg/mL	0.42 µg/mL

**Table 3.** Precision data of sitagliptin.

Concentration (µg/mL)	Mean	Standard deviation	Relative standard deviation (%)
2	5748	16	0.27
4	13,052	389.68	1.98
6	24,301	210.53	0.86

**Table 4.** Precision data of metformin.

Concentration ( $\mu\text{g/mL}$ )	Mean	Standard deviation	Relative standard deviation (%)
20	77,196	172.17	0.22
40	143527.6	964.88	0.67
60	238452.6	1146.78	0.48

**Table 5.** Recovery of sitagliptin.

Sample % level	Amount added ( $\mu\text{g/mL}$ )	Amount found ( $\mu\text{g/mL}$ )	% Recovery	Mean recovery
50%	6	5.95	99.1	
50%	6	5.86	97.6	98.2
50%	6	5.89	98.1	
100%	8	7.98	99.7	
100%	8	7.90	98.75	99.9
100%	8	8.1	101.25	
150%	10	10.1	101	
150%	10	9.9	99	99.6
150%	10	9.89	98.9	

**Table 6.** Recovery of metformin.

Sample % level	Amount added ( $\mu\text{g/mL}$ )	Amount found ( $\mu\text{g/mL}$ )	% Recovery	Mean recovery
50%	60	59.3	98.8	
50%	60	59.8	99.6	99.5
50%	60	60.1	100.1	
100%	80	79.4	99.25	
100%	80	79.1	98.8	99.38
100%	80	80.1	100.1	
150%	100	99.8	99.8	
150%	100	100.3	100.3	99.7
150%	100	99.2	99.2	

#### 4. Conclusion

A simple, selective and sensitive reverse phase HPLC analysis method was developed for the simultaneous estimation of sitagliptin and metformin in marketed formulation. The method was found economical and simple with involvement of few steps. The assay has been validated as per the ICH guideline and the results have shown that the method is sensitive, accurate and reproducible. Therefore, the developed method is found suitable for the simultaneous determination of sitagliptin and metformin in formulations.

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