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Analytical Method Development and Validation for Simultaneous Estimation of Metformin HCl and Sitagliptin RP-HPLC

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ABSTRACT

RP- HPLC method has been Development and Validation For Simultaneous Estimation for Metformin HCl And Sitagliptin in Bulk was developed using Phenomenex Gemini C18 (250 × 4.6mm), 5µ. Column as stationary phase and mobile phase: HPLC grade water (Merck GR Grade)) the flow rate was maintained at a flow rate of 1.2ml/min, the retention time of Metformin and Sitagliptin were found to be 4.285 min and 7.485 min, and detection was carried out at 240nm. The high recovery and low coefficients of variation confirm the suitability of the method for simultaneous analysis of the Sitagliptin and Metformin in bulk.

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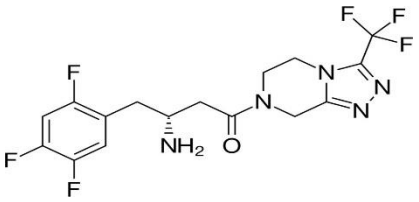
INTRODUCTION

Sitagliptin is an orally active Dipeptidyl peptidase 4 (DPP-4) inhibitor. It is a white to off-white crystalline, non-hygroscopic powder. It is soluble in water and N, N-dimethyl formamide; slightly soluble in methanol; very slightly soluble in ethanol, acetone, and acetonitrile; and insoluble in isopropanol. Metformin Hydrochloride is an Oral hypoglycemic agent. It is a white to off-white crystalline, non-hygroscopic powder. It is freely soluble in water, slightly soluble in alcohol, practically insoluble in Acetone and Methylene chloride.



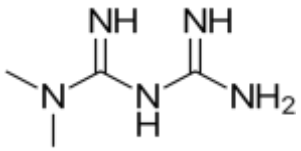


Figure No. 1: Structure of Sitagliptin **Figure No. 2: Structure of Metformin Hydrochloride**

An attempt has been made to develop a method for the simultaneous quantification of Sitagliptin Phosphate and Metformin Hydrochloride by the RP-HPLC method. The literature review regarding Sitagliptin and Metformin suggests that various analytical methods are reported for a simultaneous determination as a drug, in pharmaceutical formulation. [4]

EXPERIMENTAL

MATERIALS AND METHODS:

S. No.	Name of the working/reference standard	% Purity
1	Metformin HCL Working standard	99.65
2	Sitagliptin Working standard	99.61

Selection of Mobile Phase and its Strength:

Solution of Combination of Sitagliptin and metformin was prepared and injected into the HPLC system. The solution was analyzed using different proportion of 30:35:35, 30:40:30, 55:45, 40:40:20 v/v.

Selection of Mobile Phase pH:

Under the chromatographic conditions mentioned above the different ratios of the mobile phase were tried. The chromatograms were observed for each of the trials, out of which 30: 35:35 i.e.; 30 Buffer: 35 Methanol: 35 Acetonitrile was selected as the separation was achieved in minimum retention time.

Selection of Flow Rate:

The mobile phase consisting of buffer: methanol: acetonitrile was used and the chromatograms were recorded at flow rates of 1ml/min, 1.2ml/min. The sharpest peaks were obtained with a 1.5 ml/min flow rate.

Selection of Analytical Wavelength:

Solution of Metformin HCL and Sitagliptin was scanned in the UV region and spectrum was recorded. The solvent used was 0.02M dipotassium hydrogen phosphate and acetonitrile in the ratio 55:45. It was seen that at 260nm all compounds have good absorbance, which can be used for the estimation of compounds by HPLC.

Preparation of standard stock solutions

Weigh accurately about 50mg of Metformin, 50mg Sitagliptin working standard to a 100ml volumetric flask. Dissolve it completely and sonicate it. Makeup to 100ml mobile phase. Take 3ml from the above flask and makeup to 50ml with the mobile phase.

Accuracy: Sitagliptin and Metformin

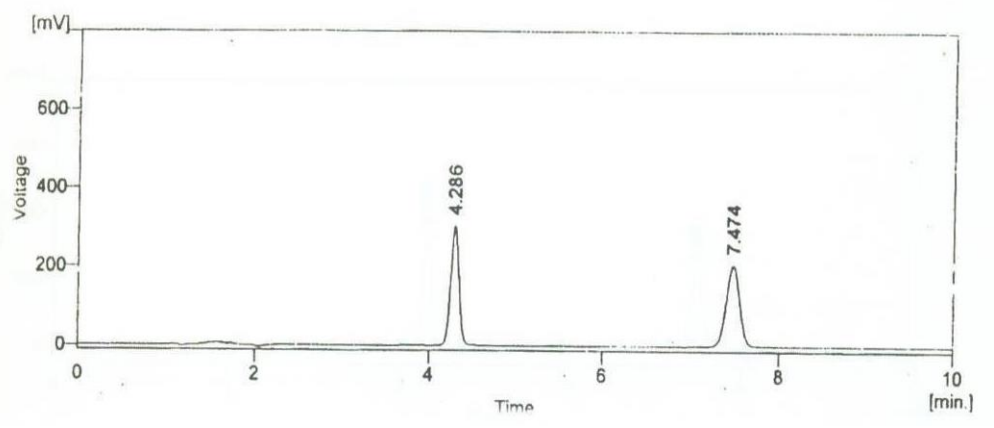


Figure No. 3: Chromatogram-27 (Metformin and Sitagliptin Accuracy 80%)

Table No. 1: Results of Accuracy for RP-HPLC Method for sitagliptin. (Accuracy 80%)

Sr. No.	Retention time (Min.)	Area (mV)	Area (%)
1	4.286	1993.980	51.9
2	7.474	1848.658	48.1
3	Total	3845.630	100.0

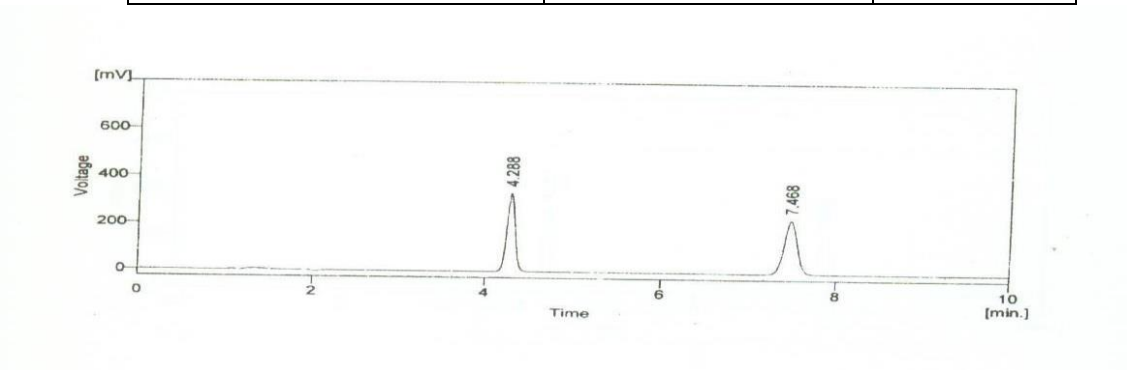
Precision: Sitagliptin and Metformin

Sr. No.	Metformin	Sitagliptin
1	2051.31	2218.08
2	2061.14	2228.22
3	2047.51	2230.72
4	2055.83	2229.53
5	2043.94	2212.8
6.	2039.57	2217.84
AVG	2049.88	2222.86
STD/%RSD	0.33/0.33	0.37/0.38

Ruggedness:

Ruggedness, according to the USP, is the degree of reproducibility of the results obtained under a variety of conditions, expressed as % relative standard deviation (RSD). These conditions include differences in laboratories, analysts, instruments, reagents, and experimental periods.

Robustness Criteria	RT of Metformin	RT of Sitagliptin
Change in flow +0.2	3.707	6.100
Change in flow -0.2	4.790	7.560
Change in wavelength by -P^H	4.27	7.44
Change in wavelength by + P^H	4.28	7.48



CONCLUSION:

RP-HPLC method was developed. It was validated for the estimation of Metformin HCL and Sitagliptin in tablet dosage form using HPLC Shimadzu Prominence with UV-Visible SPD 20A Detector and Phenominex C18 (250x4.6mm, 5µ) column, injection of 20 µl is injected and eluted with the mobile phase of dipotassium hydrogen phosphate buffer, and acetonitrile in the ratio 55:45, which was pumped at a flow rate of 1ml at 260 nm. The peak of Metformin HCL and Sitagliptin is found well separated at 4.285 and 7.485 respectively. The developed method was validated for various parameters as per ICH guidelines like Accuracy, Precision, Linearity, Specificity, Ruggedness, Robustness, LOQ, and LOD. The analytical method validation of Metformin HCL and Sitagliptin by RP HPLC method was found to be satisfactory and could be used for the routine pharmaceutical analysis of Metformin HCL and Sitagliptin.

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