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RP- HPLC Method Development and Validation for Simultaneous Estimation for Metformin and Sitagliptin in Bulk and Tablet Formulation

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Abstract : RP- HPLC method have been Development and Validation For Simultaneous Estimation for Metformin And Sitagliptin in Bulk and Tablet Formulation was developed using Grace C18 column(250nm x 4.6ID, Particle size: 5 Micron) as stationary phase and methanol : HPLC grade water (80:20%v/v, pH3.0) as mobile phase was maintained at a flow rate of 0.8ml/min, the retention time of Metformin And Sitagliptin were found to be 6.19 min and 7.42 min and detection was carried out at 254nm. The high recovery and low coefficients of variation confirm the suitability of the method for simultaneous analysis of the Sitagliptin and Metformin in bulk and tablet Formulation. The validated method was successfully used for quantitative analysis of Janumet tablet.
Key Words : Metformin, Sitagliptin, Spectrophotometric Method, RP-HPLC.

Introduction

Sitagliptin phosphate 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 in Methylene chloride. Janumet® Film-coated tablets are available for oral administration in strengths of 50 mg and 500 mg

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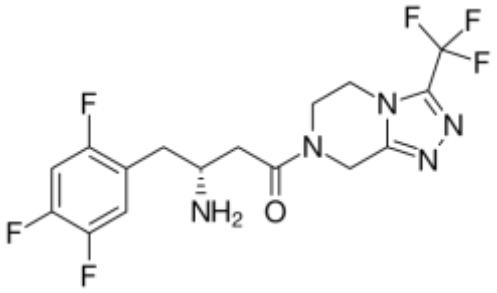
of Sitagliptin Phosphate and Metformin Hydrochloride. Each film-coated tablet of JANUMET contains the following inactive ingredients: microcrystalline cellulose, poly vinyl pyrrolidone, sodium lauryl sulfate, and sodium stearylfumarate. In addition, the film coating contains the following inactive ingredients: polyvinyl alcohol, polyethylene glycol, talc, titanium dioxide, red iron oxide, and black iron oxide.[1],[2],[3]



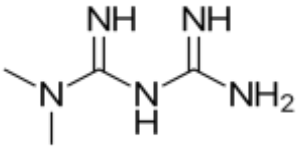


Fig. 1: Structure of Sitagliptin Phosphate **Fig. 2: Structure of Metformin Hydrochloride**

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

Experimental

Materials and methods:

Sitagliptin and Metformin were supplied by Swapnroopagenceies, Aurangabad, India as gift sample and used as such. Methanol used was spectro grade from Qualigen fine chemicals Ltd, India. Water used was HPLC grade, Potassium Dihydrogen Phosphate - AR grade, Ortho Phosphoric Acid - AR grade, Acetonitrile - HPLC grade.

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 Methanol: HPLC Grade water such as 50:50, 70:30, 80: 20% v/v.

Selection of Mobile Phase pH:

pH of HPLC grade water was adjusted to 3 using 1% orthophosphoric acid. The solution of Combination of Sitagliptin and metformin (20µg/ml) was prepared and injected into the HPLC system.

Selection of Flow Rate:

Chromatogram of solution of Combination of Sitagliptin and metformin (20µg/ml) 1.2, 1, 0.8ml/min

Selection of Analytical Wavelength:

The standard solution of Combination of Sitagliptin and metformin (20µg/ml) in mobile phase were scanned separately in the UV region of 190 to 800 nm and the overlain spectra were recorded.

Preparation of Mobile Phase:

Mobile phase was prepared by mixing 800 ml of methanol and 200 ml of HPLC grade water whose pH was adjusted to 3 using 1% orthophosphoric acid. The mobile phase was filtered through 0.2µm Supor 200 membrane filter using Vacuum Pump and ultrasonicated for 10 min.

Preparation of standard stock solutions

An accurate weighed tablet powder equivalent to about 10mg of sitagliptin & metformin was transfer into 100ml volumetric flask and sonicated for the 10min 25ml of HPLC grade methanol and made up the volume with the same solvent of 100µg/ml of Sitagliptin & metformin. The resulting solution filtered through whatman filter paper no 41 and this solution was used as Stock solution means to prepare 1000ppm of stock solution of tablet we will have to weight 0.0.300gm of tablet and dissolve it into the 10ml of solvent.

Analysis of the marketed formulation

Weigh 0.0300gm of tablet and dissolve it into the 10ml of solvent. that gives 1000ppm of drug present in the tablet. To prepare 30ppm of tablet solution , 0.3ml of stock solution was withdrawn and made up vol. up to 10ml.

Table 1: Assay of marketed formulation of Sitagliptin and metformin.

Sr. No	% Composition	Area of Standard	Area of Sample	%Assay
1	Sitagliptin	1065365	1069805	100.41
2.	Metformin	163280	163776	100.30

Validation

Accuracy: Sitagliptin and Metformin

Linearity was determined separately for Sitagliptin and Metformin by plotting peak area against concentration. From these calibration plots it was clear that response was a linear function of concentration over the ranges 200–1000 ng/mL for Sitagliptin and Metformin as shown in graphs 1, 2.

Table 2: Results of Accuracy for RP-HPLC Method for sitagliptin.

Sr. No.	Concentration	Area	Standard Deviation		Accuracy	Precision
			Mean	SD	%SD	%RSD
1	10	299104	298925.6667	172.7956404	0.05780555	0.057805555
	10	298759				
	10	298914				
2	30	1065365	1064747.667	2018.1034	0.18953818	0.189538185
	30	1066385				
	30	1062493				
3	50	1823267	1823226.333	1547.400831	0.08487157	0.084871571
	50	1824753				
	50	1821659				

Table 3: Results of Accuracy for RP-HPLC Method for Metformin.

Sr. No.	Concentration	Area	Standard Deviation		Accuracy	Precision
			Mean	SD	%SD	%RSD
1	10	69483	69296	176.626725	0.25488733	0.254887331
	10	69132				
	10	69273				
2	30	163280	163776.3333	429.8375662	0.26245402	0.0262454017
	30	164024				
	30	164025				
3	50	269635	269109.3333	459.6480538	0.17080346	0.17080346
	50	268783				
	50	268910				

Precision: Sitagliptin and Metformin

The precision of an analytical method was studied by performing intra- day and inter- day precision.

1. Intra-day Precision:

Intra-day precision was determined by analyzing the standard solution of Sitagliptin and metformin (30µg/ml) at three different time intervals on same day.

2. Inter-day Precision:

Inter- day precision was determined by analyzing the standard solution of Sitagliptin and Metformin (30µg/ml) on three consecutive days.

Table: 4. Results of Precision of sitagliptin and metformin

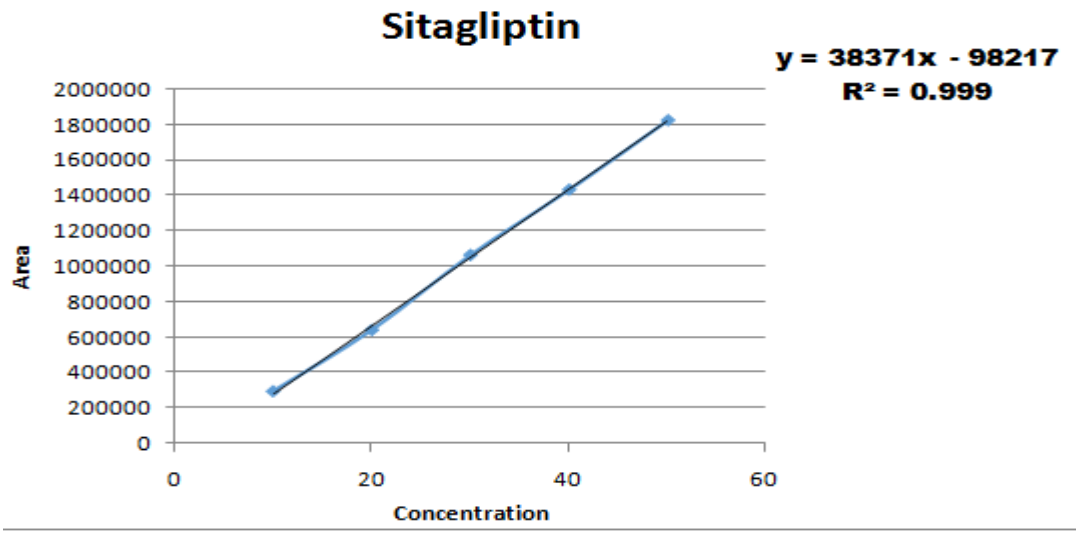
Sr No.	Precision	Sitagliptin (% RSD)	Metformin (% RSD)
1.	**Inter- Day**	0.19%	0.66%
2.	**Intra- Day**	**0.14%**	0.48%

Linearity:

The concentration of ranges 5- 50µg/ml were prepared and analyzed. From the data linearity were determined.

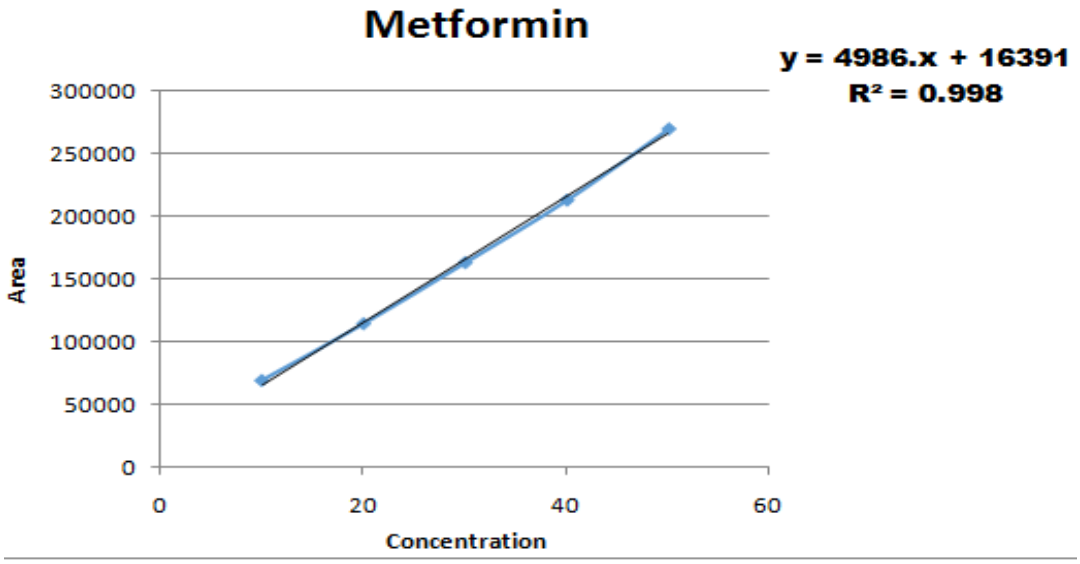
Preparation of Standard Calibration curves

Calibration curve of Sitagliptin:



1. HPLC Linearity graph for Sitagliptin

Calibration curve of Metformin:



2. HPLC Linearity graph for Metformin

Limit of Detection and Limit of Quantitation:

Detection limit and quantitation limit were determined based on the standard deviation of y - intercepts of five Calibration curve and average slope of six calibration curves as mentioned.

SITAGLIPTIN:

Limit of Detection:

$$\text{LOD} = \frac{3.3 \times \text{Std.Deviation}}{\text{Slope}}$$

$\frac{3.3 \times 2492.19}{38371}$= 0.21

Limit of Quantitation:

LOQ= $\frac{10 \times \text{ Std.Deviation}}{\text{Slope}}$

$\frac{10 \times 2492.19}{38371}$ = 0.64

Met formin :

Limit of Detection:

LOD= $\frac{3.3 \times \text{ Std.Deviation}}{\text{Slope}}$

$\frac{3.3 \times 355.37}{4986}$= 0.23

Limit of Quantitation:

LOQ= $\frac{10 \times \text{ Std.Deviation}}{\text{Slope}}$

$\frac{10 \times 355.37}{4986}$ = 0.71

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, analyst, instruments, reagents, and experimental periods.

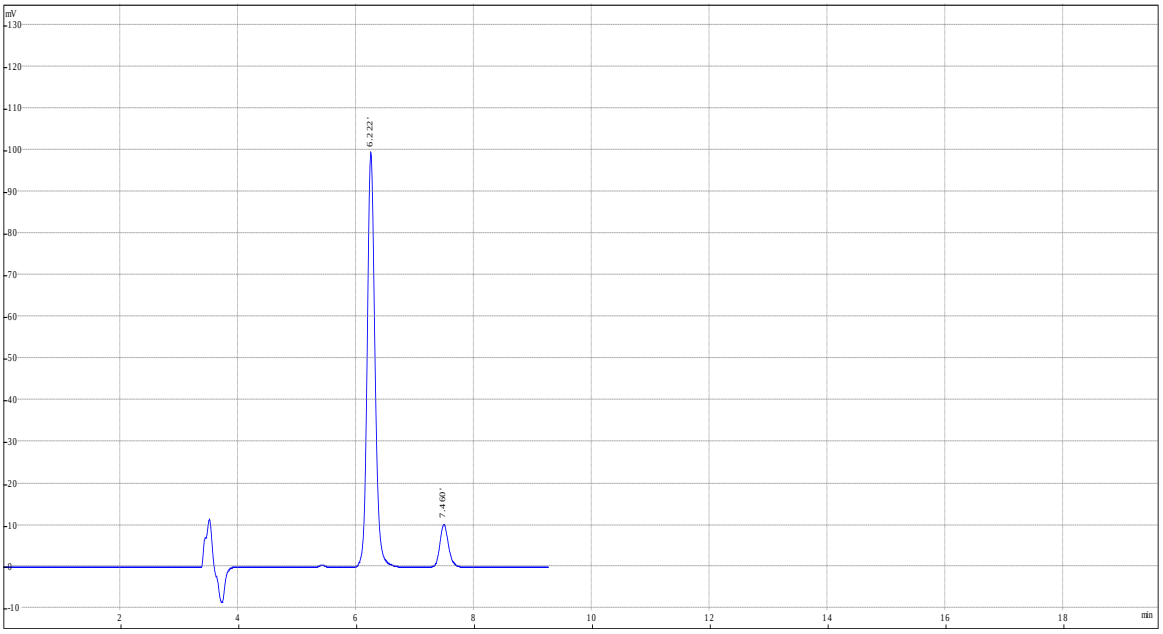
Table: 5. Ruggedness of Sitagliptin& Metformin

Sr.No	Sample	RT	Area	Resolution	T.PlateNum	Asymmetry
1	Sit	5.867	303798	3.05	6596	1.14
	Met	7.021	70548	0.00	7877	1.12
2	Sit	5.895	640954	2.83	6065	1.14
	Met	7.068	113621	0.00	7021	1.14
3	Sit	6.185	1068141	2.73	6460	1.13
	Met	7.414	165214	0.00	7874	1.13
4	Sit	6.248	1440864	3.05	6451	1.13
	Met	7.493	211320	0.00	7714	1.12
5	Sit	5.858	1825084	2.93	6472	1.13
	Met	7.019	270993	0.00	7298	1.14

% Recovery:

Result of % Recovery of Sit+Met

Sr. No	% Composition	Area of Standard	Area of Sample	% Recovery
1	Sitagliptin	1065365	1062140	99.6972
2		1432810	1436231	100.2387
3		1823267	1826746	100.1908
4	Metformin	163280	162392	99.4561
5		212965	212705	99.8779
6		269635	269879	100.0904



Chromatogram of Sit+Met in Methanol: HPLC Water (80:20 % v/v, pH 3) at flow rate of 0.8ml/min at 254nm

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