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Development and Validation for Simultaneous Estimation of Sitagliptin and Metformin in Pharmaceutical Dosage Form using RP-HPLC Method

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Abstract: A simple, accurate, specific and reliable RP-HPLC method for the simultaneous estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Pharmaceutical dosage form was developed and validated according to currently accepted ICH guidelines of analytical method validation. In the present method, SHIMADZU HPLC with UV detector LC 10 AT *VP* with analytical column PHENOMENEX Luna (C18) A 100 RP Column, 250 mm x 4.6 mm x 5µm, an injection volume of 20µl was injected and eluted with mobile phase 0.02M Potassium dihydrogen phosphate pH(4.0) : Acetonitrile (60:40) pumped at a flow rate of 1.0ml/min. Sitagliptin Phosphate and Metformin Hydrochloride were eluted at 2.718 and 1.925 min. The detection was carried out at a wavelength 252nm. The method was validated for system suitability, linearity, accuracy, precision and robustness of sample solution. The linear ranges for Metformin Hydrochloride and Sitagliptin Phosphate were 20-120µg/mL, 2-12µg/mL respectively with good recoveries i.e. 99.4% to 101.35%.
Keywords: Metformin Hydrochloride, Sitagliptin Phosphate, 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 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 stearyl fumarate. 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

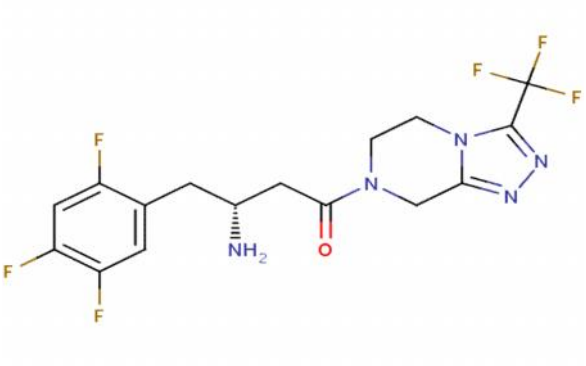
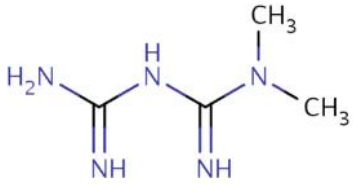


Fig. 2: Structure of Metformin Hydrochloride



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 and in various biological fluids. [4]-[9]

3. Materials And Methods

Chemicals and reagents

Mobile phase preparation

3.1. Preparation of Buffer solution:

2.87g of potassium dihydrogen phosphate is dissolved in 1000 ml of volumetric flask and make up with water and adjust the pH to 4.0 with ortho phosphoric acid. Filtered through a finer porosity membrane filter and degassed.

Mobile Phase:

Mixed Buffer and ACN in the ratio of 60:40v/v respectively and degassed by ultra sonication.

3.2. Preparation of diluent:

Diligent into consideration the solubility of the drugs in different solvents, the common diluent was selected for all the two drugs which is nothing but the Water.

3.3. Preparation of Standards and Samples

3.3.1 Preparation of Standard: (Sitagliptin & Metformin Standard)

1. Weighed accurately and transferred about 10mg of Sitagliptin working standard, 100mg of Metformin working standard taken separately in 100ml volumetric flasks.
2. Added about 80ml of diluent, sonicated to dissolve and diluted to volume with the same.
3. Filtered the solution through centrifuge.

3.3.2 Preparation of Sample Solution:

Twenty tablets were accurately weighted and average was calculated. The tablets were then crushed to obtain a fine powder. An accurately weighted quantity of tablet powder equivalent to about 100 mg of Metformin and 10mg of Sitagliptin was transferred to 100 mL volumetric flask, sonicated with 80 mL of diluent with intermediate shaking for 30 min. The volume was made up to the mark with diluent and the resulting solution was filtered.

Optimized chromatographic conditions are

Mode of operation	: Isocratic
Stationary phase	: Phenomenex Luna C18 A 100 C_{18}Column (250mm X 4.6 mm i.d.,5µ)
Mobile phase	: 0.02M Potassium dihydrogen phosphate pH (4.0): Acetonitrile
Ratio	: 60:40
Diluent	: Water
Detection wavelength	: 252 nm
Flow rate	: 1.0 ml/min
Temperature	: 25°C
Sample volume	: 20 µl

4. Method Development:

4.1. Experimental Procedure:

4.4.1. Procedure:

The chromatographic conditions were set as per the established parameter and mobile phase allowed to equilibrate with the stationary phase. Working standard solution was injected separately and chromatograms have been reproduced.

Study of system suitability parameters:

Procedure:

The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Five replicate injections of working mixed standard solution were injected and the chromatograms were recorded for the drugs and the results are shown in the table 1.

The retention times and responses of the analyte were recorded. The system suitability is evaluated by inbuilt system suitability software calculating the mean, standard deviation and coefficient of variation for the retention time and area.

5. Validation Of Proposed Method

5.1. Linearity:

Accurately measured volume of standard stock solution was diluted with diluent to get the final concentrations of sitagliptin standard as 2-12 µg/ml and metformin standard as 20-120 µg/ml respectively. Six point linearity was determined.

5.5.1 Procedure:

The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Standard solutions of different concentration were injected separately and the chromatograms were recorded.

Peak areas were recorded for each injected concentration of drugs and the calibration curves, concentration vs. peak area were constructed for the drugs.

Linearity performance parameters are depicted below. Peak areas were recorded and the graphs, concentration vs. peak area were constructed for the drugs.

5.2. Accuracy:

Accuracy of proposed method was ascertained on the basis of recovery studies performed by standard addition at different level of labeled claim (i.e. 20 to 100 % of labeled claim).

5.5.1 Standard solution:

Weighed accurately and transferred about 10mg of Sitagliptin working standard, 100mg of Metformin working standard was taken in to 100ml volumetric flask separately. Added about 80ml of water, sonicated to dissolve and diluted to volume with the same. Filtered the solution through centrifugation.

5.5.2. Sample Solution:

Accurately weighted quantity of placebo was taken in five different 100 mL volumetric flasks. To these flasks accurately weighed quantities of API equivalent to percent of 10 mg of Sitagliptin, 100mg of Metformin are added to their respective flasks. About 80mL of diluent was added to all the flasks and then sonicated for 30min with intermittent shaking. The volume was made up to mark with diluent. Filtered the solutions through centrifugation.

5.5.3 Procedure:

The chromatographic conditions were set as per the optimized parameters and the mobile phase was allowed to equilibrate with stationary phase. Five replicate injections of the standard solution and three replicate injections of each sample solution were injected separately and chromatograms were recorded. The concentration of each drug was estimated by comparing sample peak area with standard. The results of accuracy are shown in **table 2.**

5.3. Precision:

5.5.1. Standard Solution:

Sitagliptin and Metformin Standard Solution was used.

5.5.2. Sample Solution:

Twenty tablets were accurately weighted and average was calculated. The tablets were then crushed to obtain a fine powder. An accurately weighted quantity of tablet powder equivalent to about 100 mg of Metformin and 10mg of Sitagliptin was transferred to 100 mL volumetric flask, sonicated with 80 mL of diluent with intermediate shaking for 30 min. The volume was made up to the mark with diluent and the resulting solution was filtered.

5.5.3. Procedure:

The chromatographic condition were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase, five replicate injection of standard solution and each of sample solutions were made separately and the chromatograms were recorded and the results are shown in the **table 3.**

5.4. Robustness:

The sample solution of Sitagliptin and Metformin are prepared having concentration of 10 mg/ml and 100 mg/ml respectively and analyzed using different chromatographic condition as below and to show the system is stable within the chromatographic conditions of the proposed method.

a) Change in flow rate

b) Change in organic phase composition in the mobile phase (± 10%)

c) Change in pH of mobile phase (± 0.2)

i. Change in flow rate

Five replicate injections of standard solution and sample solution are injected at different flow rates. The chromatograms were recorded and the results are shown below. The results are shown in the table 4.

ii. Change in organic phase of mobile phase (± 10%)

Five replicate injections of standard solution and sample solution are injected at different concentration of organic phase (±10%) i.e. mobile phase having concentration of Buffer: ACN as (a) 600:440 and (b) 600:360. The results are shown in the table 4.

iii. Change in pH of mobile phase (± 0.2)

Five replicate injections of standard solution and sample solution are injected at different pH of buffer (±0.2) i.e. mobile phase having buffer of pH 3.8 and 4.2. The results are shown in the table 4.

5.5. Ruggedness: The ruggedness was determined by using the data obtained by the analysis performed by different analyst using different reagent, different instrument and different column. Each analyst prepared six samples of the same batch. The results obtained are given in table 5.

5.6. Sensitivity:

The limit of detection (LOD) was calculated using the following equation LOD=3.3σ/s where σ is standard deviation of y intercept of the calibration curve (n=6) and s is the slope of regression equation. The results obtained are given in table 6.

6. Results:

6.1 Figures & Tables:

Figure 1: Optimized Chromatogram Of Sitagliptin And Metformin:

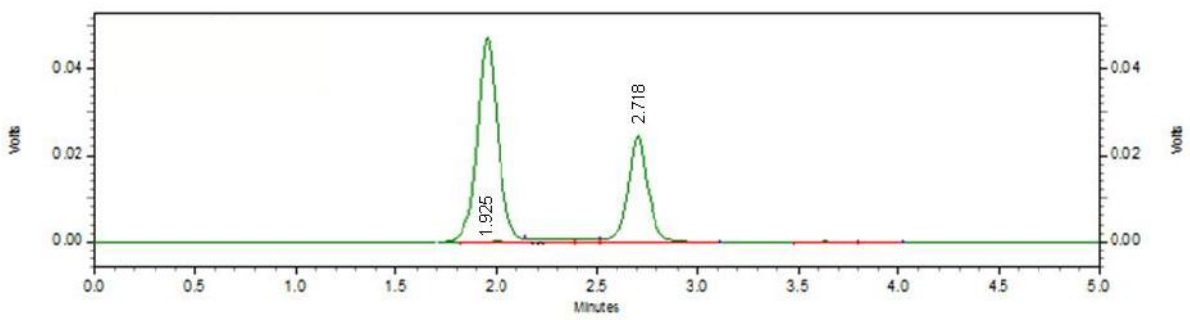


Figure 2: Linearity Of Sitagliptin:

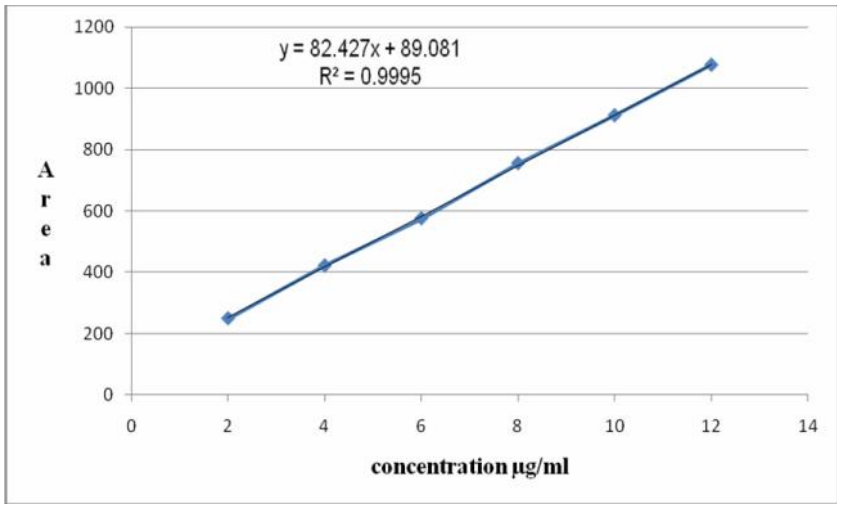


Figure 3: Linearity Of Metformin:

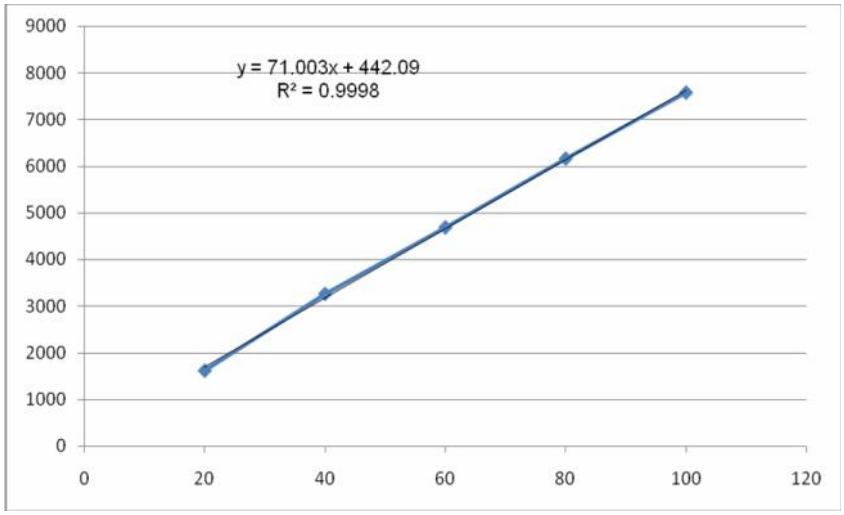


Table – 1: System suitability parameters of Sitagliptin and Metformin

Parameters	Sitagliptin	Metformin
[Area] Mean of 5 Injections ±SD %RSD	1919.879 29.82 0.15	2595.285 34.41 0.13
Retention time (min)	2.718	1.925
Theoretical plates	2410.12	5178.8
Asymmetry factor	1.42	1.16

Table – 2: Results of accuracy studies for Sitagliptin and Metformin

Spiked level of drug (%)	Amount of drug added (µg/ml)		Amount of drug found (µg/ml)		% recovery	
	Sitagliptin	Metformin	Sitagliptin	Metformin	Sitagliptin	Metformin
20	2	20	12.09	19.28	100.8	99.4
40	4	40	20.05	40.28	100.2	100.2
60	6	60	20.27	60.64	101.35	100.4
80	8	80	20.21	79.10	101.05	99.5
100	10	100	20.08	100.4	100.44	100.2

Table -3: Precision of Sitagliptin and Metformin

Sample	Assay of Sitagliptin			Assay of Metformin		
	Mean area	mg/ unit	% Label claim	Mean area	mg/ unit	% Label claim
1	1932.112	9.90	99	2606.9266	98.79	98.79
2	1924.579	9.88	98.8	2601.8090	98.014	98.01
3	1928.013	9.99	99.9	2630.7782	98.10	98.10
4	1942.091	9.83	98.35	2598.5883	99.30	99.30
5	1944.579	9.90	99	2606.9266	99.43	99.43
	Mean		99.01 ± 39.85		98.72	98.72 ± 0.57
	%RSD		0.40			0.005

Table - 4 : Results of Robustness

Experiment (Robustness)	Tailing Factor		Theoritical plates	
	Sitagliptin	Metformin	Sitagliptin	Metformin
Control	1.15	1.34	5405.72	4347.94
Flow minus	1.20	1.49	5419.82	4362.76
Flow plus	1.14	1.31	5410.98	4354.73
Column temperature plus	1.20	1.48	5439.83	4396.78
P^H minus	1.18	1.43	5890.44	4783.45
P^H plus	1.12	1.24	5189.90	4141.34
Increased organic	1.17	1.58	5388.98	4238.89
Decreased organic	1.12	1.38	5289.89	4206.34

Table – 5: Results for ruggedness of Sitagliptin and Metformin

	Assay (% Label claim)		Assay (% Label claim)	
	Sitagliptin 50mg		Metformin500 mg	
Sample	Analyst 1	Analyst 2	Analyst 1	Analyst 2
1	96.4	98.36	97.27	98.30
2	98.8	99.72	98.01	97.38
3	99.9	98.98	98.10	96.54
4	98.35	99.65	97.37	98.89
5	99	98.75	98.01	97.59
Mean	98.49 ± 1.29	99.09 ± 0.58	97.752 ± 0.39	97.74 ± 0.89
% RSD	1.30	0.58	0.40	0.91

Table - 6 :LOD & LOQ

	SITAGLIPTIN	**METFORMIN**
LOD (µg/ml)	0.3374	0.0446
LOQ (µg/ml)	1.1247	0.1488

Table - 7 : Validation Parameters

S. No	**Parameters**	**Observation**	
		Sitagliptin	**Metformin**
1	Linearity range (µg/ml)	2-12	20-120
2	Regression coefficient($r^{2)}$	0.9992	0.9995
3	Method Precision	0 .98	0.99
4	Accuracy (%recovered)	100.2.-101.35	99.4-100.4
5	Robustness (%RSD)	1.2-1.4	0.3-0.4
6	LOD (µg/ml)	0.3374	0.0446
7	LOQ (µg/ml)	1.1247	0.1488
8	Slope	82.427.0	71.003
9	Y-Intercept	89.081	442.09

6. Conclusion:

A RP-HPLC method for the simultaneous determination of Sitagliptin phosphate and Metformin Hydrochloride in Pharmaceutical formulation was developed and validated according to currently accepted ICH guidelines of analytical method validation.

In the present work, an attempt has been made to develop the method using RP HPLC method for simultaneous estimation of Sitagliptin and Metformin in combined dosage form with simple, accurate, specified with less retention times.

HPLC with UV/Vis detector with Phenomenex Luna C18 A 100, C_{18} (250×4.6×5µ) with an injection volume of 20µl is injected and eluted with the mobile phase of Potassium dihydrogen phosphate buffer P^H 4.0 : Acetonitrile (60:40), which is pumped at a flow rate of 1.0 ml/min and detected by u.v detector at 252nm. The peaks of Sitagliptin and Metformin are found well separated at 2.718 and 1.925 minutes respectively.

The proposed method was found to be rapid, accurate, precise, specific, robust and economical and complies system suitability limits. The mobile phase is simple to prepare and economical. The calibration was linear in the concentration range of 20-120 µg/ml for metformin and 2-12 µg/ml for sitagliptin respectively. The RSD indicates the method is precise and accurate, the mean recoveries were found in the range of 99-101%. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. This method is also having an advantage than reported method that the retention time of both the drugs is below 5 min and both the drugs can be assayed with the short time. Thus the method is not time consuming and can be used in laboratories for the routine analysis of combination drugs. Ruggedness of the proposed method was determined by analysis of aliquots from homogeneous slot by different analysts, using same operational and environmental parameters and the results were within the limits. The method was also specific and robust.

The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the Label value of commercial Pharmaceutical dosage form. Therefore, there is no significant difference in the results achieved by the proposed method.

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