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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF SITAGLIPTIN AND METFORMIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS.

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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.*

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INTRODUCTION:

Sitagliptin is indicated for the management of glycemic control in type 2 diabetes mellitus along with diet and exercise. Inhibition of DPP-4 by sitagliptin slows DPP-4 mediated inactivation of incretins like GLP-1 and GIP. Incretins are released throughout the day and upregulated in response to meals as part of glucose homeostasis. Reduced inhibition of incretins increases insulin synthesis and decrease glucagon release in a manner dependant on glucose concentrations. These effects lead to an overall increase in blood glucose control which is demonstrated by reduced glycosylated hemoglobin [1-2]. IUPAC name is 3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5H- [1,2,4] triazolo[4,3-a] pyrazin-7-yl]-4-(2,4,5 trifluorophenyl) butan-1-one. Molecular Formula is $C_{16}H_{15}F_6N_5O$. Molecular weight is 407.31. It is soluble in water and N, N-dimethyl formamide, slightly soluble in methanol, soluble in ethanol, acetone and acetonotrile and insoluble in isopropanol and isopropyl acetate.

Metformin is an antihyperglycemic agent of the biguanide class, used for the management of type II diabetes. Metformin is considered an antihyperglycemic drug because it lowers blood glucose concentrations in type II diabetes without causing hypoglycemia. Metformin is commonly described as an *insulin sensitizer* leading to a decrease in insulin resistance and a clinically significant reduction of plasma fasting insulin levels [3]. Metformin's mechanisms of action are unique from other classes of oral antihyperglycemic drugs. Metformin decreases blood glucose levels by decreasing hepatic glucose production (gluconeogenesis), decreasing the intestinal absorption of glucose, and increasing insulin sensitivity by increasing peripheral glucose uptake and utilization. It is well established that metformin inhibits mitochondrial complex I activity, and it has since been generally postulated that its potent antidiabetic effects occur through this mechanism. The above processes lead to a decrease in blood glucose, managing type II diabetes and exerting positive effects on glycemic control [4,5]. IUPAC name 3-(diaminomethylidene)-1,1-dimethylguanidine.
Molecular weight is 129.16. Molecular formula is $C_4H11N5.HCl$. It is freely soluble in water; slightly soluble in alcohol; practically insoluble in acetone and in methylene chloride.

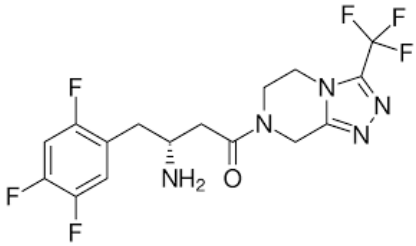

Figure 1: Structure of Sitagliptin

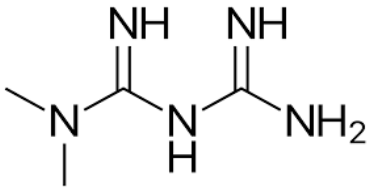

Figure 2: Structure of Metformin

The literature survey revealed that There are really few approaches reported in the literary works for evaluation of Sitagliptin and Metformin alone or in combination with various other drugs in the pure form as well as drugs formulations by RP-HPLC [6-11]. In view of the demand for an appropriate, cost-effective RP-HPLC method for routine analysis of Sitagliptin and Metformin synchronized evaluation of in pharmaceutical dose type. Attempts were made to establish easy, precise, accurate as well as cost-efficient logical method for the estimate of Sitagliptin and Metformin. The recommended approach will be validated according to ICH guidelines. The objective of the recommended work is to establish a brand-new, simple, delicate, exact and economical logical method as well as recognition for the Synchronized evaluation of Sitagliptin and Metformin in pharmaceutical dose kind by utilizing RP-HPLC. To verify the established method based on ICH standards for the desired analytical application.

MATERIALS AND METHODS:

Chemicals and Reagents: Sitagliptin and Sitagliptin were Purchased from Sun Pharma India Limited. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 252 nm with column Phenomenex Luna C18 A 100 C_{18}Column (250mm X 4.6 mm i.d.,5µ), dimensions at 25^0C temperature. The optimized mobile phase consists of 0.02M Potassium dihydrogen phosphate pH (4.0): Acetonitrile (60 40). Flow rate was maintained at 1 ml/min.

Preparation of solutions:
Mobile phase preparation

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.

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.

Preparation of Standards and Samples

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.

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.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Sitagliptin and Metformin peaks

RESULTS AND DISCUSSION

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Phenomenex Luna C18 A 100 C_{18}Column (250mm X 4.6 mm i.d.,5µ), the mobile phase of composition 0.02M Potassium dihydrogen phosphate pH (4.0): Acetonitrile was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Table 1: System suitability parameters

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

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Sitagliptin and Metformin in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Table 2: Assay results for Sitagliptin and Sitagliptin

	Label Claim (mg)	% Assay
Sitagliptin	10	100.13
Metformin	100	100.12

Figure 3: Standard chromatogram

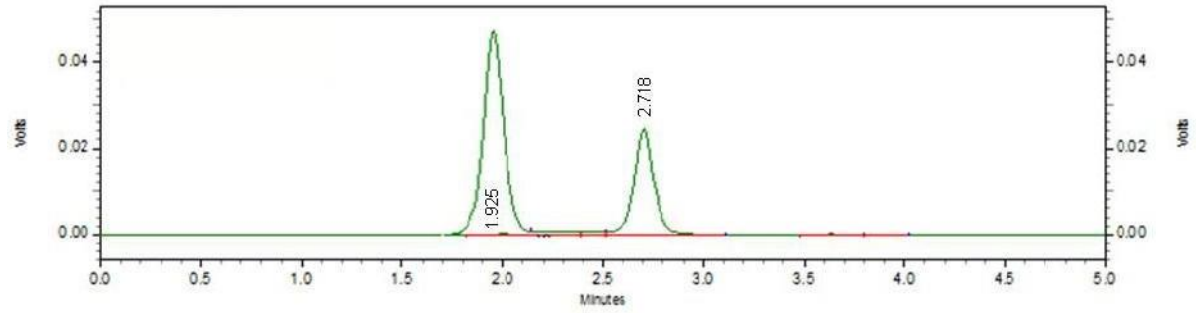

Figure 4: Sample chromatogram

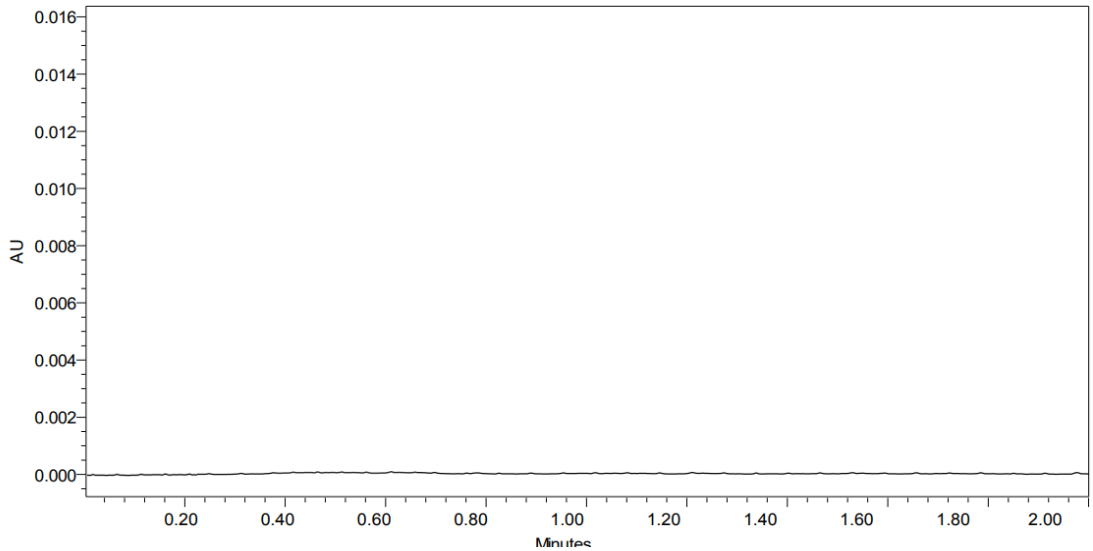

Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 50 ppm to 150 ppm and 50 ppm to 150 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

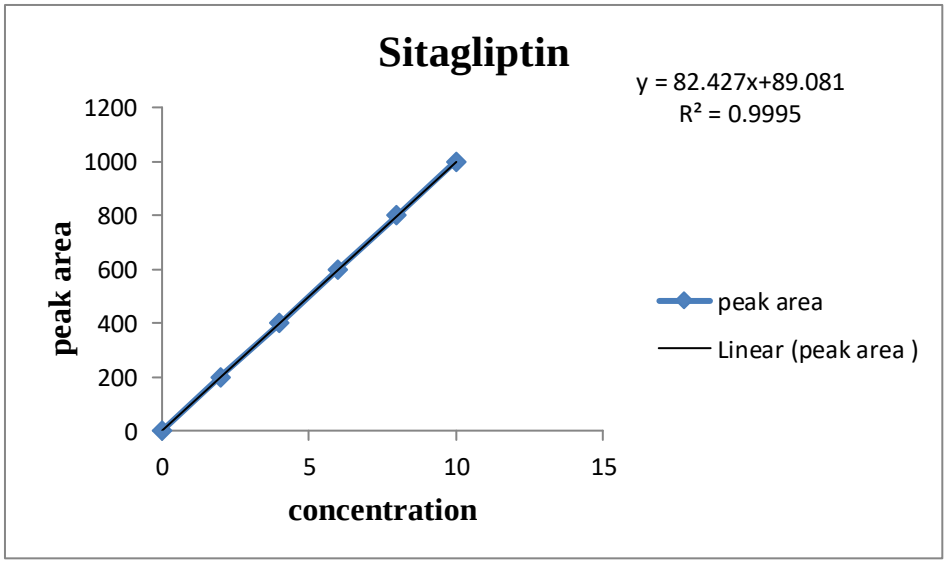

Figure 6: Linearity graph for Sitagliptin

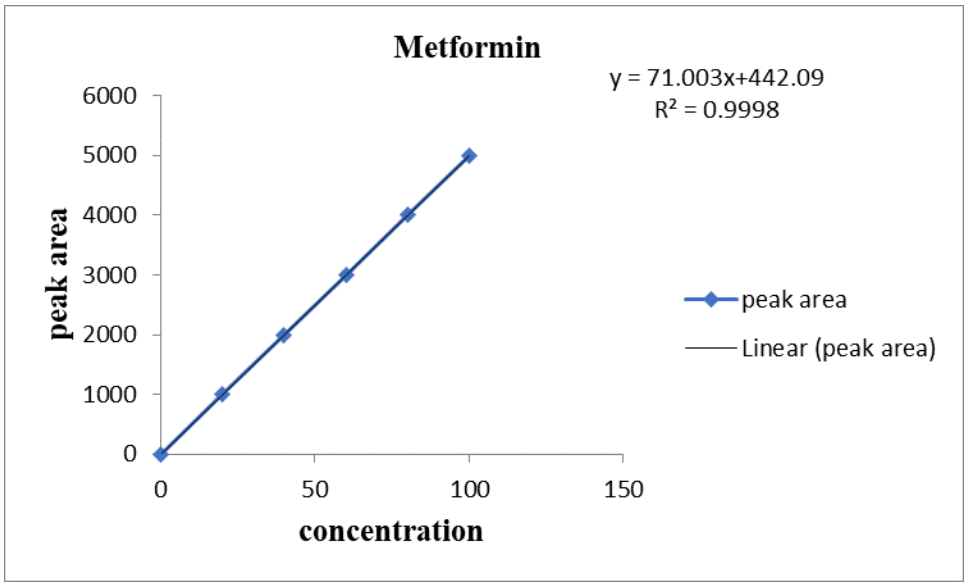

Figure 7: Linearity graph for Metformin

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at 20%, 40%, 60%, 80%, 100% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Sitagliptin and Sitagliptin and calculate the individual recovery and mean recovery values. The results are shown in table 3.

Table 3: Showing accuracy results 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

Precision Studies: precision was calculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 4.

Table 4: Precision results for 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

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different Analyst. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 5.

Table 5: Ruggedness results 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

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The results are shown in table 6.

Table 6: Flow variation results for Sitagliptin and Metformin

Experiment (Robustness)	Tailing Factor		Theoretical 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

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 7.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Table 7: LOD, LOQ of Sitagliptin and Metformin

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

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. 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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