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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF METFORMIN AND SITAGLIPTIN IN BULK AND TABLET DOSAGE FORM BY RP-HPLC

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Abstract:
A new RP-HPLC method for the quantitative determination of Metformin and Sitagliptin was developed and validated as per ICH guidelines. The drugs were injected into Kromasil C18 (4.6 x 250mm, 5µm), maintained at ambient temperature and effluent monitored at 254 nm.The mobile phase consisted of OPA (0.1%) : Acetonitrile (40% : 60%) The flow rate was maintained at 1.0 ml/min. The calibration curve for Metformin and Sitagliptin were linear from 125-7500µg/ml and 12.5-75µg/ml respectively (r^2 for Metformin = 0.999,r^2 for Sitagliptin = 0.999).The proposed method was adequate, sensitive, reproducible, accurate and precise for the determination of Metformin and Sitagliptin in bulk and tablet dosage form.

Keywords: *Metformin and Sitagliptin, Validation.*

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INTRODUCTION:

Metformin is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It is also used in the treatment of polycystic ovary syndrome. Limited evidence suggests metformin may prevent the cardiovascular disease and cancer complications of diabetes. Metformin is in the biguanide class. It works by decreasing glucose production by the liver and increasing the insulin sensitivity of body tissues. It occurs as a white to almost white powder which is freely soluble in water. Sitagliptin is an oral antihyperglycemic (antidiabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. It is (3R)-3-amino-1-[3-(trifluoromethyl)-5H,6H,7H,8H- [1,2,4] triazolo[4,3-a] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan-1-one. It is white, soluble in water. It is a highly selective DPP-4 inhibitor, which is believed to exert its actions in patients with type 2 diabetes by slowing the inactivation of incretin hormones, thereby increasing the concentration and prolonging the action of these hormones. It is used as an adjunct to diet and exercise to improve glycemic control in patients with type 2 diabetes mellitus [1-7] .Various analytical methods have been reported for the estimation of Metformin and Sitagliptin including spectrophotometric methods and HPLC. HPLC is the most widely used technique for the estimation of Metformin and Sitagliptin in human plasma, saliva, cerebrospinal fluid, and human blood cells, as well as for studying the drug metabolites in the urine. The suggested HPTLC and HPLC methods for assay of Metformin and Sitagliptin are quite expensive and need complex and sophisticated instrumentation. The present research work describes a HPLC and UV spectrophotometric method for estimation of Metformin and Sitagliptin in API and its pharmaceutical preparation [8-13]. The present method aims at developing a simple, accurate and precise RP-HPLC method for the estimation of Metformin and Sitagliptin in bulk and tablet dosage form.

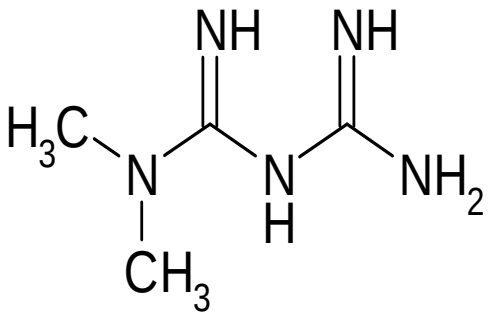

Fig 1: Chemical structure of Metformin

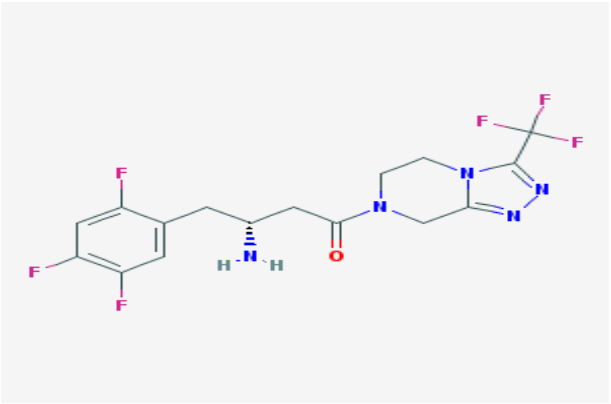

Fig 2: Chemical structure of Sitagliptin

MATERIALS AND METHODS:

Chemicals and solvents:

The reference sample of Metformin and Sitagliptin was obtained as a gift sample from Rhodes Pharmaceuticals, India. HPLC grade water (prepared by using 0.45 Millipore Milli –Q) was procured from Standard Reagents, Hyderabad. HPLC grade Acetonitrile, Methanol, Ortho phosphoric acid was bought from Merck, Mumbai.

Instrumentation:

An Alliance model 2996 equipped with a UV spectrophotometer having PDA detector for finding out the λmax values of the drugs was used throughout this study. An Kromasil ODS 18(250×4.6, 5 mm) column was employed for the method development. The chromatographic system was monitored by Empower software. Analytes were monitored by UV detection at 254 nm using an isocratic mode with Orthophosphoric acid (0.1%): Acetonitrile in the ratio 40:60 using water and methanol as diluent which was used as mobile phase. The flow rate was set at 1.0 ml/min and effluent was monitored at 254 nm.The temperature and run time were maintained at 25°C and 6 min. respectively. Solubility of the compounds was enhanced by sonication on an ultrasonicator (BVK Enterprises).

Selection of mobile phase:

The objective of this experiment was to optimize the assay method for estimation of Metformin and Sitagliptin based on the literature survey. Various mobile phases were tested to select the best possible system. The various mobile phases used included Water: Methanol (50:50), Water: Acetonitrile (50:50), 60% OPA: 40% Acetonitrile, **50**%

OPA: 50% Acetonitrile, 45% OPA: 55% Acetonitrile, Better peak resolution and adequate retention time were obtained with the ratio of 40% OPA (0.1%): 60% Acetonitrile.

Preparation of Mobile Phase:
The mobile phase was prepared by taking 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water. The mobile phase was then degassed using Ultra-Sonicator to remove dissolved gases and the resultant mobile phase was filtered through a 0.45 µm membrane filter under vacuum. HPLC grade Acetonitrile was used .The mobile phase composition selected was 40% OPA (0.1%) : 60% Acetonitrile.

Preparation of Standard solution:
Standard solution was prepared by accurately weighing 50 mg of Metformin and 5mg Sitagliptin and transferring them into a 100 ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10 mins. and then made upto volume with the mobile phase. The resultant mobile phase was filtered through a 0.45 µm membrane filter under vacuum. From this 1 ml of solution was taken & made upto 10 ml with mobile phase. The solution was sonicated for about 10 mins. and then made upto volume with the mobile phase.

Preparation of Sample solution: Sample solution was prepared by accurately weighing 5 tablets and calculating the average weight of each tablet. From that calculated weight 50mg of Metformin and 5mg Sitagliptin were taken and transferred them into a 100 ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10 mins. and then made upto volume with the mobile phase. The resultant mobile phase was filtered through a 0.45 µm membrane filter under vacuum. From this 1 ml of solution was taken & made upto 10 ml with mobile phase. The solution was sonicated for about 10 mins. and then made upto volume with the mobile phase.

Validation:
Prior to validation studies blank solution was injected and chromatogram was noted. Optimized conditions maintained where both the drugs were eluted with good retention time and peak area which was shown in the fig 4.

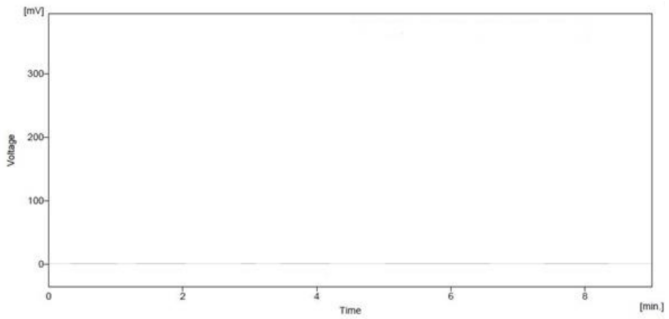

Fig 3: Blank chromatogram

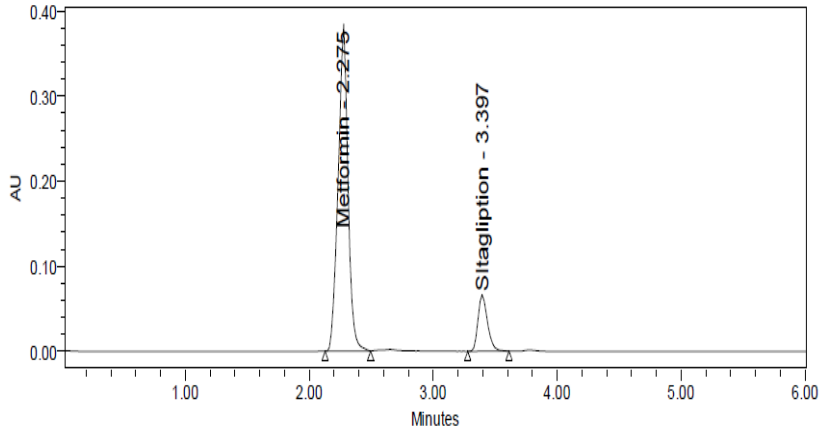

Fig 4: Optimized Chromatogram

Linearity: The linearity of the method was established by determining the absorbance of different concentrations of Metformin and Voglibose over a range of 125-750µg/ml and 12.5-75µg/ml respectively.

Table 1: Linearity data of Metformin and Sitagliptini

Metformin		Sitagliptin	
Conc (µg/ml)	**Peak area**	**Conc (µg/ml)**	**Peak area**
0	0	0	0
125	544751	12.5	89983
250	1088329	25	181763
375	1675511	37.5	276554
500	2207899	50	357366
625	2687299	62.5	450608
750	3255239	75	545511

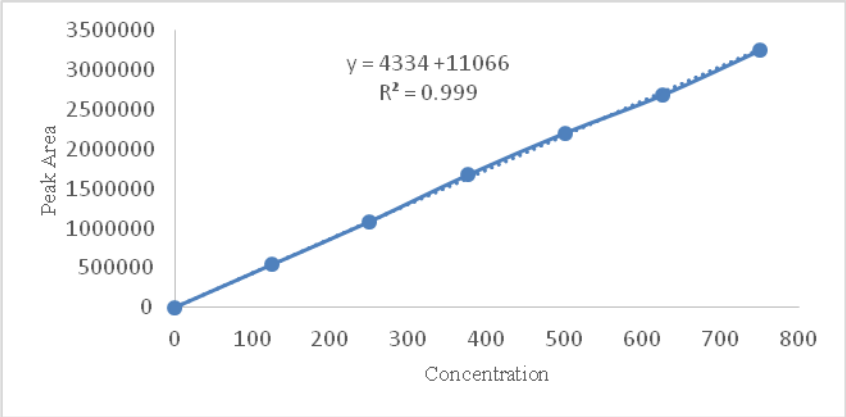

Fig 5: Calibration curve of Metformin

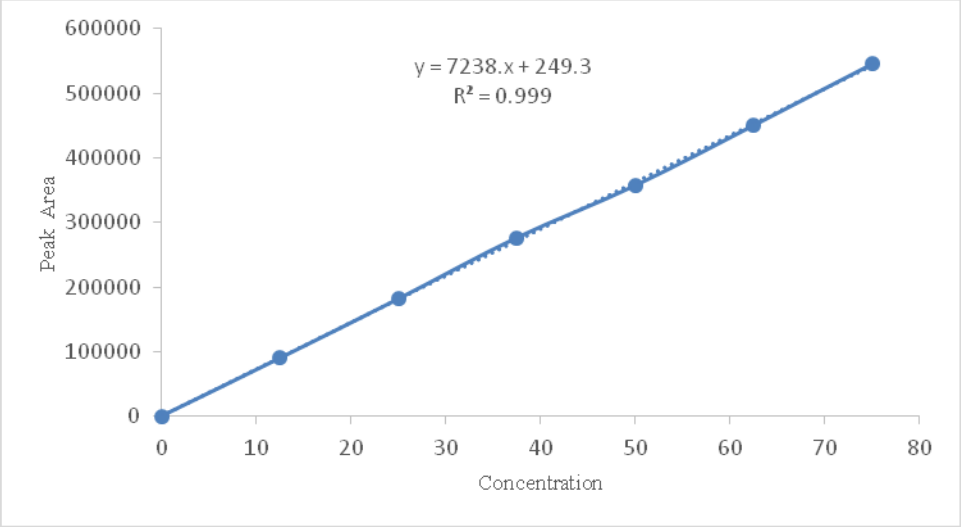

Fig 6: Calibration curve of Sitagliptin

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by analyzing the samples were carried out by analyzing the measured concentration and the added concentration of the drug. Each sample was injected thrice .The percent recoveries of the drugs were estimated.

Table 2: Accuracy data of Metformin

% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %Recovery
50%	250	248.95	99.58	100.38%
	250	251.02	100.41	
	250	252.64	101.06	
100%	500	503.24	100.65	
	500	496.77	99.35	
	500	505.01	101.00	
150%	750	755.92	100.79	
	750	746.91	99.59	
	750	757.21	100.96	

Table 3: Accuracy data of Sitagliptin

% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %Recovery
50%	25	24.57566	98.30	99.85%
	25	25.11283	100.45	
	25	24.86981	99.48	
100%	50	50.28774	100.58	
	50	49.65994	99.32	
	50	50.62858	101.26	
150%	75	74.81067	99.75	
	75	75.33071	100.44	
	75	74.31454	99.09	

Precision: Precision is one of the important factors which determine the reliability of an analytical method. The precision of the developed method was tested and was found to be suitable. Both method precision and system precision were performed and are given in table 4,5.

Table 4: Method precision data of Metformin and Sitagliptin

S. No	Area of Metformin	Area of Sitagliptin
1.	2072226	347868
2.	2051006	346145
3.	2100103	351020
4.	2047099	350578
5.	2099101	347400
6.	2125498	352590
Mean	2082506	349267
S.D	30935.2	2490.9
%RSD	1.5	0.7

Table 5: System precision data of Metformin and Sitagliptin

S. No	Area of Metformin	Area of Sitagliptin
1.	2146527	350454
2.	2112967	349579
3.	2132942	351921
4.	2119699	352996
5.	2110469	352071
6.	2113964	351757
Mean	2122761	351463
S.D	14158.07	1231.54
%RSD	0.7	0.4

Robustness: The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like volume of injection, wavelength which may differ but the responses were still within the limits of the assay.

Table 6: Robustness data of Metformin and Sitagliptin

S.no	Condition	%RSD of Metformin	%RSD of Sitagliptin
1	Flow rate 1.1ml/min	0.1	0.8
2	Flow rate 1.3ml/min	0.6	0.3
3	Mobile phase 35B:65A	0.4	0.3
4	Mobile phase 45B:55A	0.1	0.98
5	Temperature 25°C	0.1	0.9
6	Temperature 35°C	0.6	0.0

LOD and LOQ:

LOD: Limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental condition.

The LOD may be expressed as: **LOD = 3.3 σ / S**

LOQ: Limit of quantitation (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

The LOQ may be expressed as: **LOQ = 10 σ / S**

Table 7: LOD and LOQ of Metformin and Sitagliptin

Molecule	LOD	LOQ
Metformin	0.08	0.25
Sitagliptin	0.06	0.19

Assay: Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out.

Table 8: Assay data of Metformin and Voglibose combination marketed formulation

S.no	Standard Area	Sample area	% Assay
1	2146527	2102730	98.8
2	2112967	2113936	99.3
3	2132942	2134875	100.3
4	2119699	2141102	100.6
5	2110469	2135069	100.3
6	2113964	2147457	100.9
Avg	2122761	2129195	100.00
Stdev	14158.07	17177.2	0.81
%RSD	0.7	0.8	0.81

DISCUSSION:

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and economical RP-HPLC method. It was successfully applied for the determination of Metformin and Sitagliptin in pharmaceutical dosage forms without the interferences of other constituents in the formulations. Different mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was done based on

peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Orthophosphoric acid: Acetonitrile (40:60) with 1.0 ml/min flow rate was quite robust. The optimum wavelength for detection was 254 nm at which better detector response for drug was obtained. The average retention time for Metformin and Sitagliptin were found to be 2.276 and 3.397.The calibration was linear in concentration range of 125-750 mcg/ml for Metformin and 12.5-75 mcg/ml for Sitagliptin . The low values of % RSD indicate the method is precise and accurate. Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. These results show the accuracy and reproducibility of the assay. Robustness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions, the % RSD reported was found to be less than 2%. The LOD values of Metformin and Sitagliptin was found to be 0.08mcg/ml & 0.06mcg/ml respectively. The LOQ values of Metformin and Sitagliptin were 0.25mcg/ml & 0.19 mcg/ml respectively. 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 formulation. There was no significant difference in the results achieved by the proposed method.

CONCLUSION:

The proposed method for the assay of the popular anti- diabetic drugs Metformin and Sitagliptin in the commercially available tablet formulation is simple, accurate, economical, and rapid. It can be easily adopted for routine quality control for monitoring the assay in the API, in-process samples, and the finished tablet formulation.

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