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

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ABSTRACT

A simple, accurate, rapid and precise method is developed for the quantitative simultaneous determination of Metformin and Sitagliptin in combined pharmaceutical dosage form. The method was based on HPLC separation of two drugs in reverse phase mode using C_{18}, (250 X 4.6 mm), 5µ by using Potassium Di hydrogen phosphate buffer (pH 4.0) and Methanol in the ratio of 60:40 v/v was pumped through column at a flow rate of 1.0 ml/min and detection wavelength was set at 210 nm. The retention time was 2.39 min for Metformin and 3.37 min for Sitagliptin respectively. The % RSD of Metformin and Sitagliptin were found to be 0.8 and 0.4 respectively. The % recovery was obtained as 100.43 and 100.50 for Metformin and Sitagliptin respectively. The accuracy and reliability of the method was assessed by evaluation of linearity, precision (intra-day and inter-day % RSD >2), accuracy (98-102%), specificity, LOD, LOQ values in accordance with ICH guidelines. The developed method is applicable for routine quality control analysis of selected combined dosage forms.

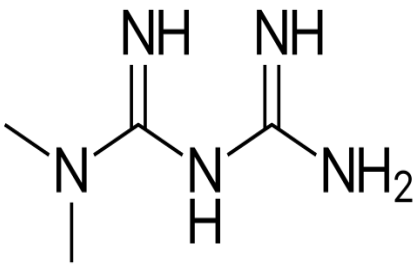
KEYWORDS: RP-HPLC, Metformin and Sitagliptin.

1. INTRODUCTION

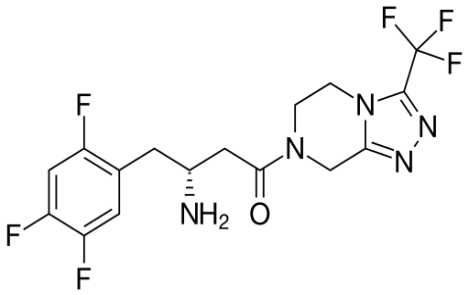
Development of simple and reproducible analytical methods for estimation of multicomponent drugs is very important part of quality control and for social awareness which is established in present work.[1]

Metformin[3] is mainly used for the treatment of type 2 diabetes. Metformin's main effect is to decrease liver glucose production. It also increases insulin sensitivity, which increases peripheral glucose uptake. Metformin decreases high blood sugar, primarily by suppressing liver glucose production. The average patient with type 2 diabetes has three times the normal rate of gluconeogenesis[2] metformin treatment reduces this by over one-third.

Sitagliptin[4] is an oral anti-hyperglycemic (anti diabetic) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase-4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal By preventing GLP-1 and GIP inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas.[2]



METFORMIN



SITAGLIPTIN

2. MATERIALS AND METHODS

2.1. Chemical and Reagents

Metformin and Sitagliptin were kindly gifted by Derex Labs Pvt Ltd, Hyderabad certified to contain 99.9% and 99.6% purity respectively. The drugs were used without further purification. All the solvents used in analysis were of HPLC grade. Istamet Tablets (label claim 50 mg of Sitagliptin and 500 mg of Metformin) of Sun pharma was used in analysis.

2.2. HPLC method

Instrument

LC system used consists of Waters HPLC having Empower Software with 2695 separation module having PDA detector with universal loop injector of injection capacity 20 µL. The column used was YMC C_{18} Column, 5µ (250× 4.6 mm) at ambient temperature. Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously.

Optimised Chromatographic conditions

The mobile phase having Potassium Di hydrogen phosphate buffer (pH 4.0) and Methanol in the ratio of 60:40 v/v was selected because it was found that it ideally resolve the peaks with retention time (RT) 2.39 min and 3.38 min for Metformin and Sitagliptin respectively. Wavelength was selected by scanning all standard drugs over a wide range of wavelength 200nm to 350nm. Both the components showed reasonably good response at 210 nm.

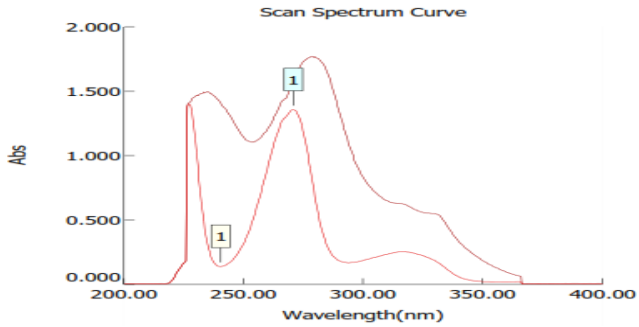


Fig 1: Overlain UV Spectrum of Metformin and Sitagliptin

Preparation of Phosphate Buffer

Accurately weigh 1.732g of Potassium Dihydrogen Ortho phosphate was taken in a 500ml volumetric flask, dissolved and diluted to 500ml with HPLC water and the volume was adjusted to pH 4.0 with Ortho Phosphoric Acid.

Preparation of Mobile Phase

Accurately measured 600 ml (60%) of above buffer and 400 ml of Methanol (HPLC Grade) (40%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Standard Solution Preparation

Accurately weigh and transfer 100 mg of Metformin and 10 mg of Sitagliptin working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Metformin and Sitagliptin peaks and calculate the %Assay by using the formulae.

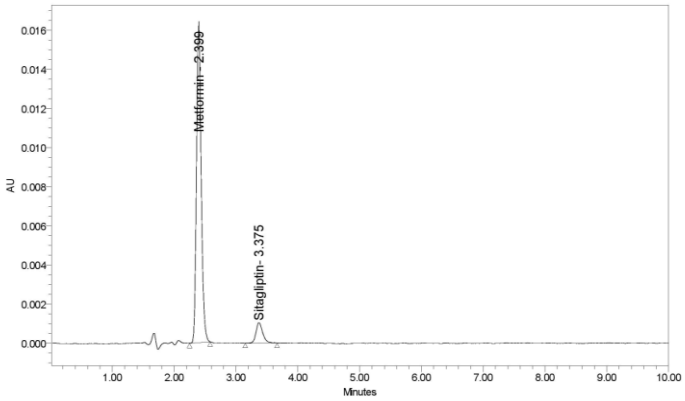


Fig 2: Chromatogram of Standard solution 1. Met (Rt 2.39), 2.Sit (Rt 3.37) at 210 nm.

Pharmaceutical Sample Solution (from Formulation)

Sample Solution Preparation

Accurately weigh and transfer 100 mg of Metformin and 10 mg of Sitagliptin working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

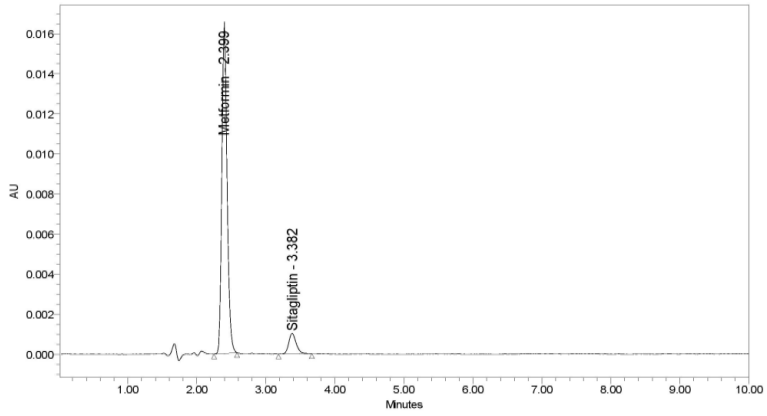


Fig 3: Chromatogram of Sample solution* 1.Met *(Rt 2.39), 2.*Sit *(Rt 3.38)

2.6. Recovery studies

To check the accuracy of sample by the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 50, 100 and 150% level. From the total amount of drug found, the percentage recovery was calculated. The results are reported in Table.

Analysis Data of Formulation

PARAMETER	**HPLC**	
	MET	**SIT**
Label claim (mg)	500	50
Drug found	500.43	50.05
% Accuracy	98-102%	

RESULTS AND DISCUSSION

HPLC

Preparation of Calibration Curves by HPLC

In a series of 10 ml volumetric flask several dilutions of MET (500-2500 µg/ml) and SIT (50-250 µg/ml) were prepared using mobile phase as solvent. Each solution was injected into HPLC system and the chromatograms were recorded. The peak areas of both drugs were calculated and the respective calibration curves were plotted against ratio of area under curve and concentration of drug.

The equations of the regression lines obtained are

For MET: $R^2 = 0.999$ for SIT: $R^2 = 0.999$

HPLC Method Validation

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, Specificity, precision, limit of detection, limit of quantitation.

1. LINEARITY

The linearity of the proposed HPLC method for determination of Metformin and Sitagliptin was evaluated by analysing a series of different concentrations of standard drug. In this study five concentrations were chosen, ranging between 50-250 µgml^{-1} of Sitagliptin and 50-2500 µgml^{-1} of Metformin. Each concentration was repeated three times. The linearity of the calibration graphs was validated by the high value of correlation coefficient, slope and the intercept value. A linear relationship was obtained for Metformin in the range of 50-2500 µgml^{-1} and Sitagliptin in the range of 50-250 µgml^{-1} respectively

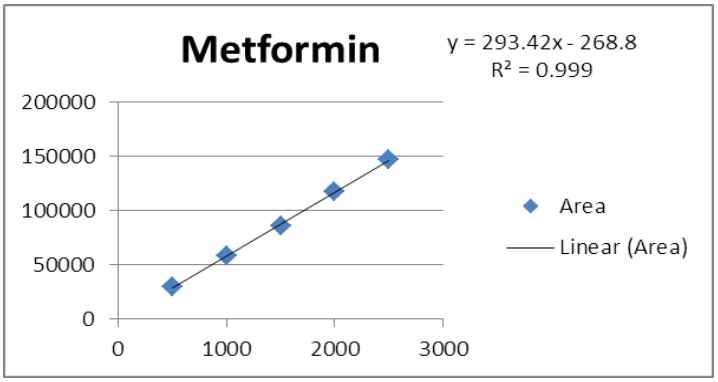


Fig 4: Linearity curve of MET

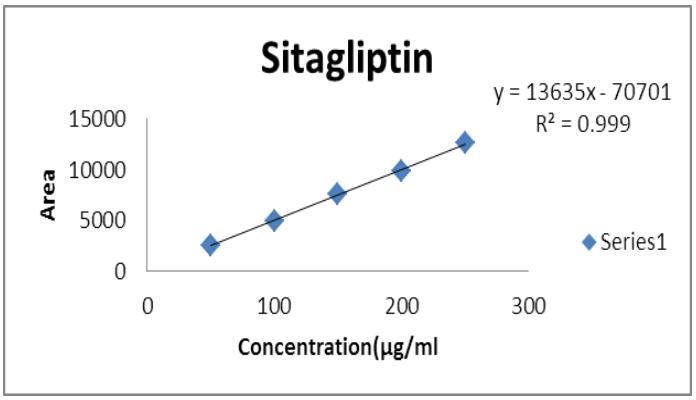


Fig 5: Linearity curve of SIT

Precision

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample.

Precision was demonstrated by repeatability and intermediate precision measurements of peak area and peak symmetry parameters of HPLC method for each title ingredients. The repeatability (within-day in triplicates) and intermediate precision (for 2 days) were carried out at five concentration levels for each compound. Triplicate injections were made and the obtained results within and between the days of trials were in acceptable range. The value of %RSD for MET and SIT were found to be less than 2 indicates that the developed method is precise.

Table 1: Precision Results for Metformin and Sitagliptin

Injection	Area for Metformin	Area for Sitagliptin
Injection-1	87799	7524
Injection-2	86973	7519
Injection-3	86232	7524
Injection-4	87604	7581
Injection-5	85975	7558
Injection-6	87018	7565
Average	86933.8	7545.2
Standard Deviation	723.5	26.2
%RSD	0.8	0.3

Accuracy

Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its linearity range. Accuracy was performed in three different levels, each level in triplicate for MET and SIT using standards at 50%, 100% and 150%. Each sample was analysed in triplicate for each level.

The mean recoveries were found in the range of 98 – 102%, by which we can say the method was accurate.

Table 2: The accuracy results for Metformin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	43148.6	50	50.04	100.08	100.43
100%	86625.0	100	100.46	100.46	
150%	130313.3	150	150.13	100.75	

Table 3: The accuracy results for Sitagliptin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	3818.7	5	5.04	100.75	100.50
100%	7587	10	10.01	100.08	
150%	11447	15	15.10	100.67	

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

It is calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

Specifity

Volume of 20 µL of working placebo sample solution was injected into the chromatograph and the chromatogram was recorded and presented below.

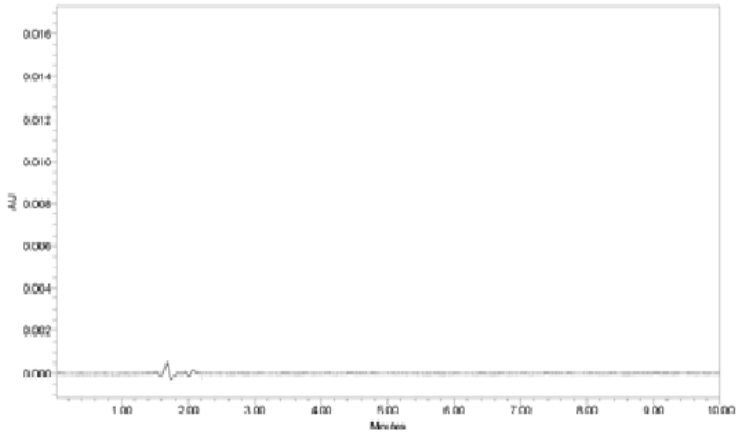


Fig 6: Blank Chromatogram

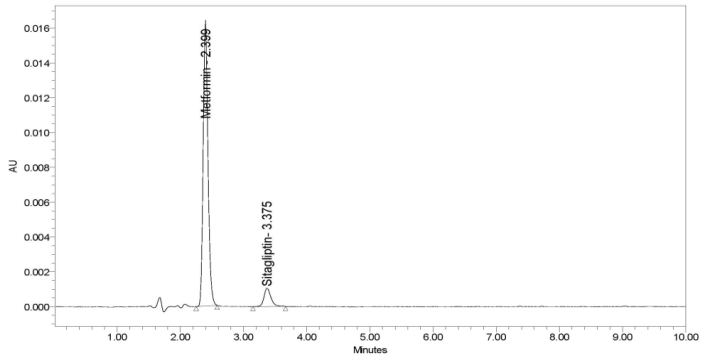


Fig 7: Drug Chromatogram

Table 2: Validation Parameters

Validation Parameters		HPLC	
		METFORMIN	**SITAGLIPTIN**
Calibration range (µg mL^{-1})		**500-2500**	**50-250**
Linearity Correlation coefficient(R^2)		**0.999**	**0.999**
Precision (%RSD)	Interday	**0.8**	**0.3**
	Intraday	**0.8**	**0.4**
Accuracy	Amount labelled (mg)	**500**	**50**
	Amount found (mg)	**500.43**	**50.05**
	%Recovery ±RSD	**100.43±0.41**	**100.5±0.69**
LOD (µV)		**175**	**174**
LOQ(µV)		**579**	**580**
Tailing Factor		**1.17**	**1.32**
Theoretical plates		**3215**	**3468**

CONCLUSION

The methods described for simultaneous estimation of Metformin and Sitagliptin are found to be simple, sensitive, accurate, precise, rapid and economical. Hence method could be successfully employed for routine analysis of Metformin and Sitagliptin in their combined dosage form.

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