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DEVELOPMENT AND VALIDATION OF RP HPLC METHOD FOR DETERMINATION OF METFORMIN AND SITAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple and rapid reversed phase-high performance liquid chromatographic method was developed for simultaneous determination of Metformin and Sitagliptin in Tablet Dosage form. The elution was done with a mobile phase of Water: Methanol (60:40) on Intersil-BDS C_{18} column (250 × 4.6 mm, 5 µm particle size). The wavelength detector was set at 258 nm. Retention times for Metformin and Sitagliptin were around 2.869 min, 3.942 min respectively. The reliability and analytical performance of the proposed HPLC procedure were statistically validated according to the respect of linearity, ranges, precision, accuracy, repeatability, reproducibility, detection and quantification limits. Linear ranges were established between 20-80 µg/mL for both the drug. The LOD and LOQ for Metformin were found to be 0.663, 1.92 and for Sitagliptin were found to be 0.405, 1.228 respectively. The described High Performance Liquid Chromatography method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form

INTRODUCTION

Metformin, chemically 1-carbamimidamido-N, N dimethyl methanimidamide (Fig. 1) is a biguanide antihyperglycemic agent used for treating non insulin dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake.[1-2]. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Metformin may induce weight loss and is the drug of choice for obese NIDDM patients.[3] When used alone, metformin does not cause hypoglycaemia; however, it may potentiate the hypoglycaemic effects of sulfonylurea and insulin. Its main side effects are dyspepsia, nausea and diarrhoea. Metformin decreases fasting plasma glucose, postprandial blood glucose and glycosolated hemoglobin (HbA1c) levels, which are reflective of the last 8-10 weeks of glucose control. Metformin may also have a positive effect on lipid levels. [4-5]

Sitagliptin is a new oral hypoglycaemic of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. This enzyme-inhibiting drug is to be used either alone or in combination with metformin or a thiazolidinedione for control of type 2 diabetes mellitus. The drug works to competitively inhibit a protein/ enzyme, dipeptidyl peptidase 4 (DPP-4), that results in an increased amount of active incretins (GLP-1 and GIP), reduced amount of release of glucagon (diminishes its release) and increased release of insulin.[6-8] Several analytical methods based on UV, [9-12] HPLC, [13-14] and HPTLC [15] were reported for the determination of Metformin. Few analytical methods based on UV, [16-17] RP-HPLC, [18] were reported for the determination of Sitagliptin. Simultaneous determination of Metformin and Sitagliptin in bulk and tablet dosage form were reported by using spectrophotometric, [19] spectroflourometric[20] and HPLC[21] methods. However very few HPLC methods were reported for the simultaneous estimation of Metformin and Sitagliptin in tablet dosage form.

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The objective of this study was to develop a simple, specific, accurate, precise, sensitive RP-HPLC method for the determination of Metformin and Sitagliptin in bulk and pharmaceutical dosage forms. This method was validated in accordance with ICH guidelines. [22]

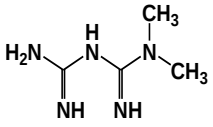


Fig 1: Metformin

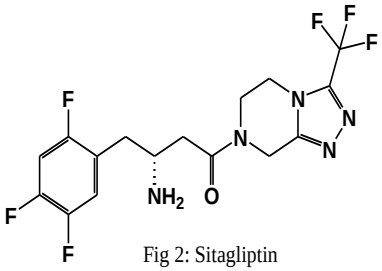


Fig 2: Sitagliptin

MATERIALS AND METHODS

Instruments

Chromatographic separation was performed using HPLC-WATERS Model NO.2690/5 equipped with PDA detector. Intersil C18 column (250×4.6 mm, 5µm particle size) was used.

Reagents and materials

All chemicals substances were of analytical reagent grade and solvents were of HPLC grade.

Chromatographic conditions

Chromatographic separation was performed on a Chromosil Intersil C18 column (250 × 4.6 mm, 5 µm particle size) . The mobile phase consisted of water and methanol in ratio 60: 40 were filtered before use through a 0.45 µ membrane and degassed for 10 min. The flow rate was 1 mL/min and the column temperature was maintained at 27°C.The volume of injection was 20 µl. The UV detector was set up at 258 nm.

Preparation of standard stock solution

Accurately weigh and transfer 100 mg of Metformin and 10 mg of Sitagliptin working standards into a 100 ml clean dry volumetric flask, add about 10 ml of mobile phase and make volume up to the mark with the mobile phase. Further pipette out 1 ml from above stock solution into 10 ml volumetric flask and dilute up to the mark with mobile phase.

Preparation of sample solutions

Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to 50 mg of Sitagliptin and 500 mg of Metformin of the tablet formulation was weighed accurately and transferred to a 100 ml volumetric flask. In RP-HPLC, to this solution 50 ml mobile phase was added and sonicated for 10 min and then final volume was made up to the mark with mobile phase. The resulting solution was filtered through Whatmann filter paper no 41, and the filtrate was appropriately diluted to get desired concentration of Sitagliptin and Metformin which was required for analysis.

RESULTS AND DISCUSSION

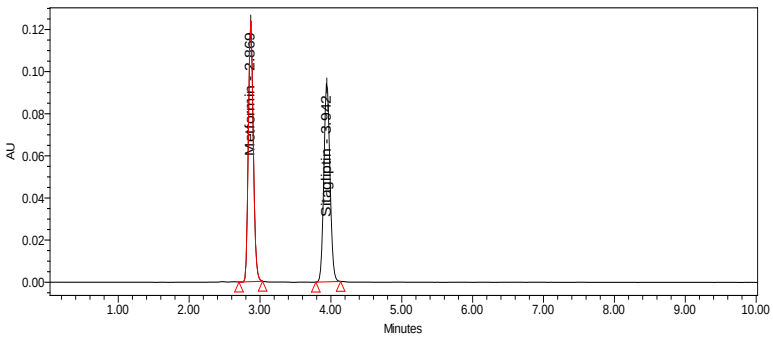
Method optimisation

A simple reverse phase high performance liquid chromatography method was developed for the determination of Metformin and Sitagliptin in pure form and in pharmaceutical formulations using Chromosil Intersil C18 column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of water and methanol in ratio 60:40. The mobile phase was chosen after several trials to reach the optimum stationary /mobile phase matching. The flow rate is 1.0ml/min. The average retention times under the conditions described were 2.869 min, 3.942 min for Metformin and Sitagliptin respectively (Table 1, Fig.3).

Table 1: Optimised Method

Parameters	Method
Stationary phase (column)	Inertsil -BDS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : water(40:60)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	258 nm
Drug RT (min)	2.8min for Metformin and 3.9for Sitagliptin

Fig. 3: Optimised Chromatogram



System Suitability

The resolution, capacity factor, theoretical plates/meter, Rt values and peak symmetry were calculated for the standard solutions. The values (Table 2) obtained demonstrated the suitability of the system for the analysis of the above drug combinations.

Linearity

The linearity of the method was determined by Preparing serial dilutions of minimum 5 concentration of standard stock solutions each in duplicate. Take the average area of each injection and plot the graph of average peak area versus actual concentration of each solution in µg/ml. Linearity ranges were found to be 20-80 µg/mL for both the drugs (Fig. 4,5).

Table 2: System suitability

Parameters	Metformin	Sitagliptin	Acceptance Criteria
USP Tailing	1.07	1.59	NMT 2.0
USP Plate count	9478	10768	NLT 2000
% RSD of peak Areas	0.01	0.04	NMT 2.0
Retention time	2.868	3.942	----

Table 3: Accuracy data

Accuracy level	Mean recovery of Metformin %	Mean recovery of Sitagliptin %
Accuracy 50%	99.69	100.06
Accuracy 100%	99.83	100.04
Accuracy 150%	99.97	100.02

Accuracy and precision

The accuracy of the method was determined by recovery experiments which were carried out and the percentage recovery and % relative standard deviation was calculated. From the data obtained, recoveries of standard drugs were found to be accurate (Table 3).The precision was carried out for System precision, Method precision and Intermediate precision (Table 4).

Specify of the method.

In formulations, chromatograms with some additional peaks were observed which may be due to excipients present in the formulations. These peaks however did not interfere with the standard peaks .The results revealed that the peak is free from interferences, which shows that the HPLC method is specific.

Table 4: Precision

Analyte	System precision		Method precision		Intermediate precision	
	Peak area ± SD	***% RSD***	***Peak area ± SD***	***% RSD***	***Peak area ± SD***	***% RSD***
Metformin	2705542 ± 739.0046	0.027314	2701851 ± 729.2689	0.026991	2706167 ± 2306.932	0.085247
Sitagliptin	1744318 ± 8241.164	0.472458	1731322 ± 5988.879	0.345914	1744608 ± 8392.59	0.481059

Fig. 4: Linearity of Metformin

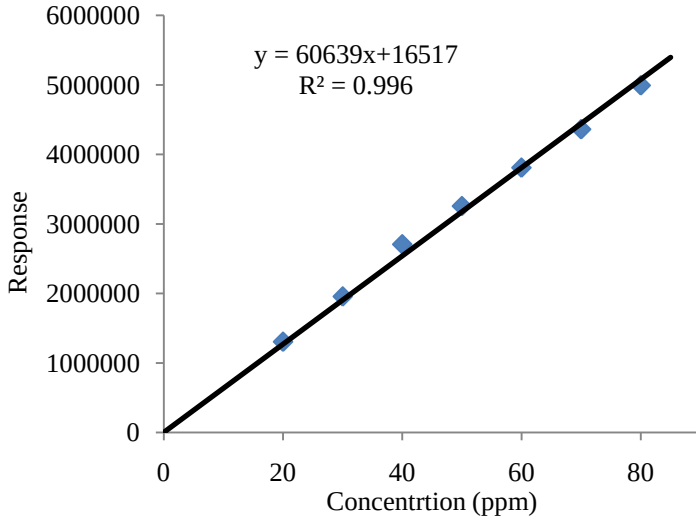


Fig. 5: Linearity of Sitagliptin

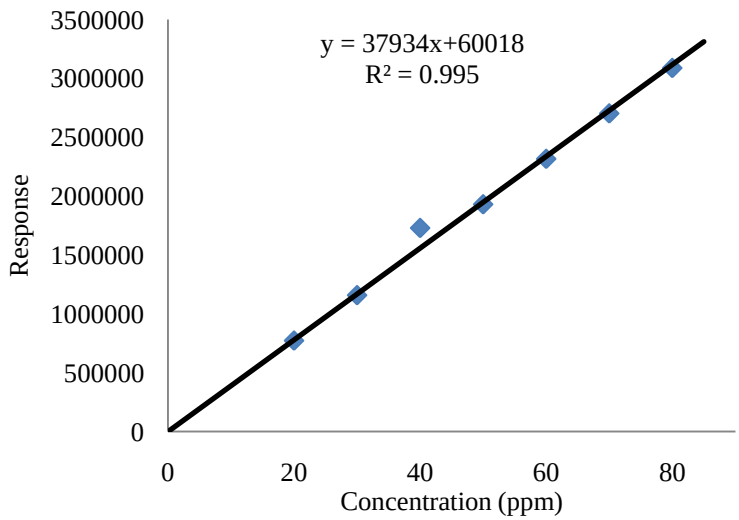


Fig 6: Flow rate for 0.8 ml/min

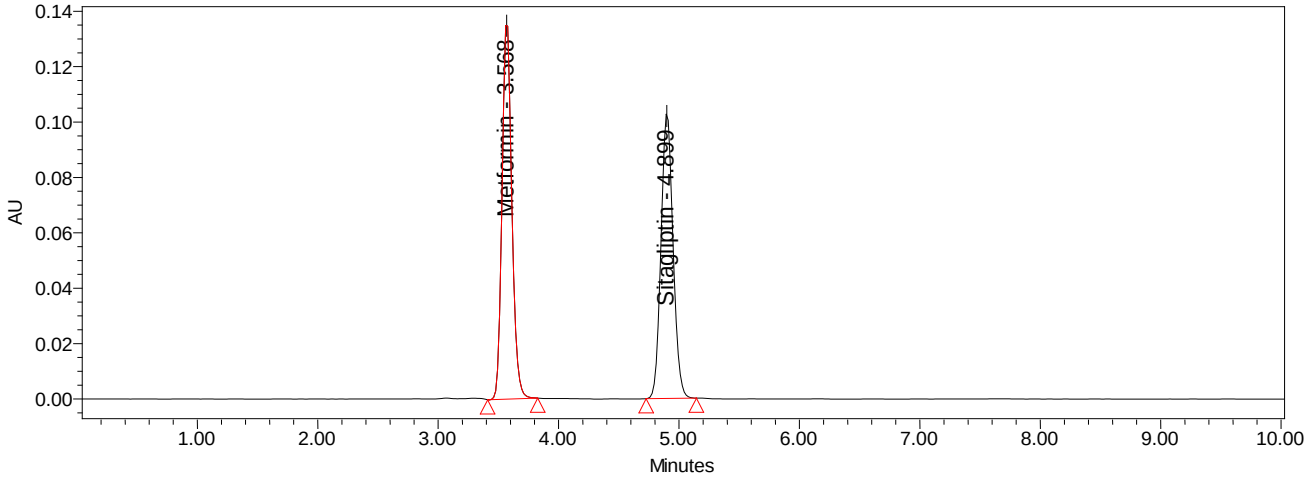


Fig 7: Flow rate for 1.2 ml/min

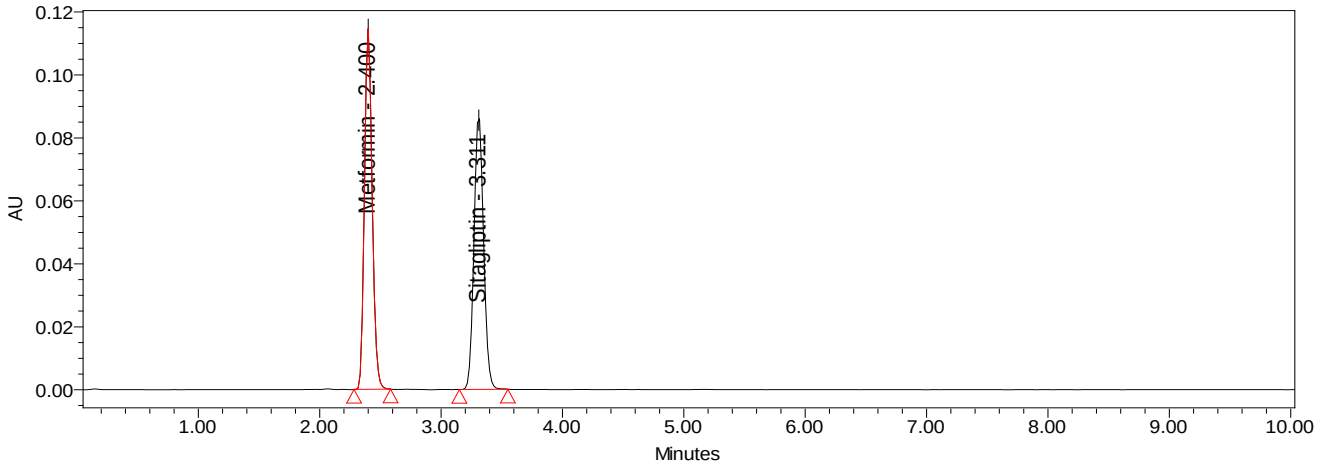


Table 5: Robustness

Parameters	% RSD	
	Metformin	Sitagliptin
0.8ml/min	0.11	0.06
1.2ml/min	0.35	0.09

Quantification Limit

The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (1:3) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (1:10). The LOD of Metformin and Sitagliptin are found to be 0.633µg/ml and 0.405µg/ml respectively. The LOQ of Metformin and Sitagliptin are found to be 1.92µg/ml 1.23µg/ml respectively.

Robustness

The robustness of the method was studied by changes in the method like alteration in flow rate (0.2 ml/min of set value i.e. 0.8 ml/min and 1.2 ml/min), detection are evaluated (Table 5, Fig 6,7).

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of Metformin and Sitagliptin and from pure pharmaceutical formulations. The mobile phase is simple to prepare and the run time was less than 5min which consumes only less than 5ml of mobile Phase shows that the method was economical.

The sample recoveries in all formulations were in good agreement with their respective label claims suggested non-interference in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Metformin and Sitagliptin in combined dosage forms. The simplicity ensures that the RP-HPLC method can be applied for estimation of Metformin and Sitagliptin in tablet dosage forms.

Since the good separation and resolution of the chromatographic peaks, the method was found to be accurate, precise, linear, robust and rugged.

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