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[Research article]

Method Development and Validation for Simultaneous estimation of Metformin Hcl and Sitagliptin by RP-HPLC in Tablet Dosage Form

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ABSTRACT

A simple, accurate, precise and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous estimation of Metformin Hydrochloride and Sitagliptin Phosphate in pure and tablet formulation. The proposed method is based on the separation of the two drugs in reversed-phase mode using zodiac C_{18} (250×4.6 mm, 5 µm particle size). The optimum mobile phase consisted of phosphate buffer : acetonitrile in the ratio of 55:45 v/v (Phosphate buffer pH 5.8 was adjusted with sodium hydroxide) was selected as a mobile phase, flow rate of 1.0 ml/min and UV detection was set at 244 nm. The retention times were 2.1 and 4.90 min for Metformin Hydrochloride and Sitagliptin Phosphate respectively. The method was validated according to ICH guidelines. It was found to be accurate and reproducible. Linearity was obtained in the concentration range of 75-175 µg/ml for Metformin Hydrochloride and 7.5-17.5 µg/ml Sitagliptin Phosphate. Mean percent recovery of samples at each level for both drugs were found in the range of 99.70% for Metformin Hydrochloride and 99.40s % for Sitagliptin Phosphate. The proposed method can be successfully applied in the quality control of bulk and pharmaceutical dosage forms.

Keywords: Metformin Hydrochloride, Sitagliptin Phosphate, HPLC, Validation.

INTRODUCTION

Metformin hydrochloride (MET) (Fig. 1) chemically, N,N-di methyl imido carbon imidicdiamide. It is a biguanide drug well known as antidiabetic drug, the mechanism of action of metformin is simulates glycolysis in peripheral tissue[1]. Sitagliptin phosphate(STG) (Fig. 2) chemically, 7-[(3R)-3-amino-1-oxo-4-(2,4,5-triflurophenyl]-5,6,7,8-tetrahydro-3-(trifluromethyl)-1,2,4-triazole [4,3] pyrazoline phosphate(1:1) monohydrate. It is a novel hypoglycemic drug that belongs to dipeptidyl-peptidase 4 inhibitor class which stimulates glucose-dependent insulinrelease[2,3]. Recently the combination of two drugs has been recommended in the treatment of diabetes mellitus to improve glycemic control[4]. This combination proved to be effective in controlling the metabolic syndrome and resulted in significant weight loss, reversal of insulin resistance, islet and adipocyte hypertrophy and achieved hepatic steatasis. According to literature survey few spectrophotometric[5-7,] HPLC[8,9] and HPTLC[10] methods have been reported for the determination of MET in single and in combination with other drugs. Analytical methods are reported for the determination of STG by

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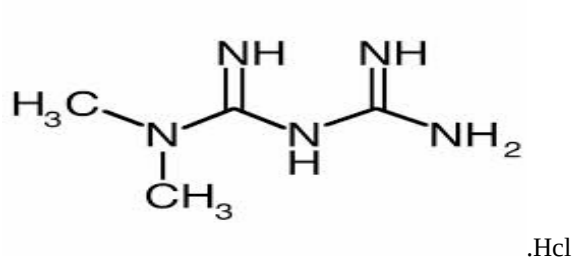
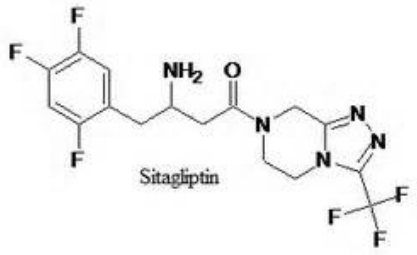
spectrophotometric[11,12] and HPLC have been reported. Simultaneous determination of MET and STG in bulk and tablet dosage form were reported by using spectrophotometric[13,14] spectroflourometric [15] and HPLC[16,17] methods. However very few HPLC methods were reported for the simultaneous estimation of MET and STG in tablet dosage form. The aim of present work was to develop and validate a sensitive HPLC method that can be applied for simultaneous estimation of MET and STG.



.Hcl

Figure 1. Structure of Metformin hydrochloride



$.H_3PO_4.H_2O$

Figure 2. Structure of Sitagliptin phosphate.

MATERIALS AND METHODS

Instrumentation

Chromatography was performed with A HPLC system (Shimadzu) Series Software (Prominence SPINCHROM) HPLC systems provided with Hamilton Syringe, auto sampler and UV-Visible detector. All HPLC systems were equipped with a column compartment with temperature control and an on-line degasser. Data acquisition, analysis, and reporting were performed by Empower (Shimadzu) chromatography software.

Reagents and chemicals

The reference samples of MET HCL and SITP were provided as gift samples from Chandra lab, Hyderabad. HPLC grade Acetonitrile, HPLC grade Methanol, HPLC grade Potassium dihydrogen orthophosphate, HPLC grade Ortho phosphoric acid, HPLC grade Ammonium acetate and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial tablets (JUNMET: 500mg Metormin + 50mg of Sitagliptin) were purchased from the local pharmacy.

PREPARATION OF SOLUTIONS

Preparation of mobile phase

0.295gms potassium dihydrogen phosphate and 0.0545 gms of Di potassium hydrogen phosphate in 100ml water adjust pH 5.8 with dilute phosphoric acid : Acetonitrile 55:45 v/v

Standard stock solution preparation

Weigh and transfer 125 mg of Metformin working standard and 12.5 mg of Sitagliptin working standard into 100 mL volumetric flask, add 60 mL of diluent and sonicate to dissolve and dilute to volume with diluent.

Working Standard preparation

Transfer 10 ml of standard stock solution into 100 ml volumetric flask and dilute to volume with diluent.

Sample Preparation

Finely grind pre weighed 20 tablets. Transfer grinded Sample quantitatively equivalent to 125 mg of Metformin and 12.5 mg of Sitagliptin in to 100 mL volumetric flask add 60 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through 0.45µ filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

ANALYTICAL METHOD DEVELOPMENT

Initially method development work as started by taking UV-Visible spectra from 200-400nm of Metformin Hcl and sitagliptin standard solutions .By observing the overlapping spectra of standard solutions λ max 244nm was taken for trials to develop HPLC method.

The spectrum was given in Fig-3

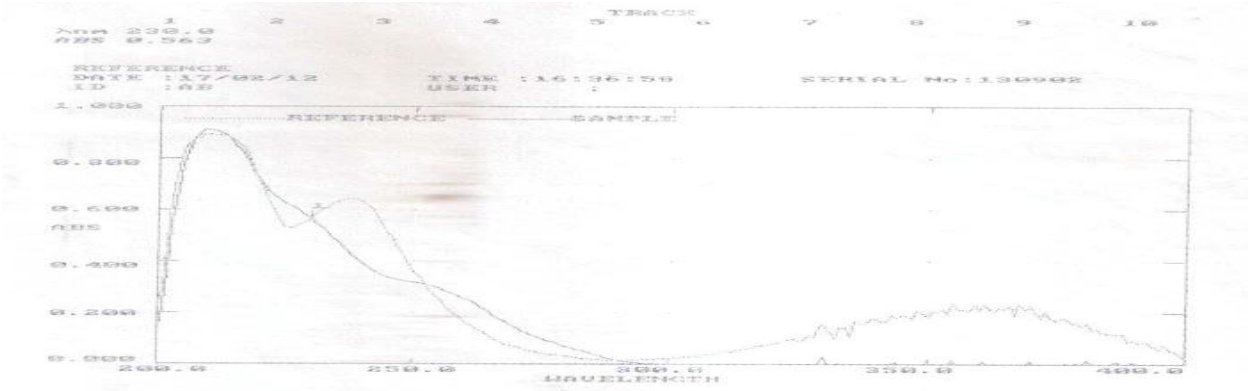


Fig.No:3 Isobestic point of Metformin Hcl and Sitagliptin

Table:1 OPTIMISED CHROMATOGRAPHIC CONDITIONS

OPTIMISED CHROMATOGRAPHIC CONDITIONS		
Mode of separation		Isocratic elution
Mobile phase		P^H 5.8 KH_2PO_4+K_2HPO_4buffer: ACN-(55:45)
Column		ZODIAC C_{18}, 250X 4.6 mm, 5µ,
Flow rate		1.0 ml/min
Detector wavelength		244 nm
Injection volume		20µl
Oven temperature		30°C
Run time		6min
Retention Time	**Metformin**	2.1
	Sitagliptin	4.9

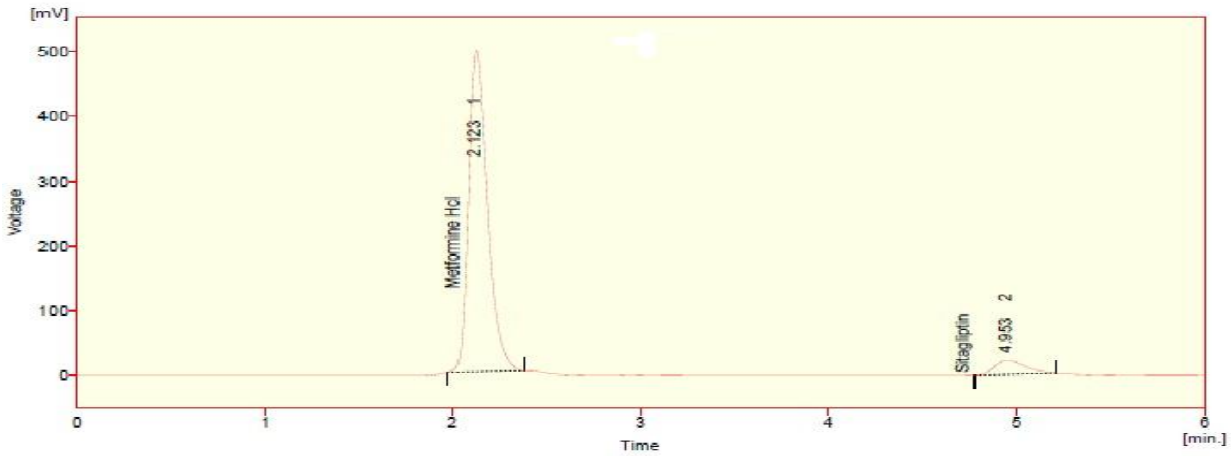


FIG :4 Optimised chromatogram of Metformin and Sitagliptin

METHOD VALIDATION PARAMETERS

The method was validated in accordance with ICH guidelines[17]. The parameters assessed were linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, reproducibility and robustness.

Linearity

Five different concentrations of the mixed standard drugs of MET and STG were prepared for linearity studies and injected into system (n=5). The response was measured as peak areas. Each

concentration was prepared from individual stock solution. The peak areas were plotted against concentrations to obtain the calibration curve.

Accuracy

The accuracy was carried out by adding known amounts of each analyte corresponding to three concentration levels (105,130, and 155%) of the labeled claim to the excipients. At each level, three determinations were performed and the accuracy results were expressed as percent analyte recovered by the proposed method.

Precision

The precision of analytical method is the degree of agreement among the individual test results, when the method is applied repeatedly to multiple sampling of homologous samples. The precision of the method was checked by repeatability of injection, repeatability (intra-day), intermediate precision (inter-day) and reproducibility. Injection repeatability was studied by calculating the percentage relative standard deviation (%RSD) for six determinations of peak areas of MET and STG.

Detection limit and quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to Equation 1 & 2, respectively.

LOD = 3.3 X SD/S…….. (1)

LOQ = 10 X SD/S…….. (2)

Where SD is the standard deviation of response (peak area) and S is the average of the slope of the calibration curve.

Robustness

Robustness was assessed by introducing small changes in the mobile phase composition and flow rate measuring the effects of result.

Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation products and related substances.

RESULTS

Method validation

Linearity

The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 75-175 µg/ml and 7.5-17.5 µg/ml for MET and STG respectively. Linear regression data for the calibration curves are given in Table 2.

Accuracy

The % mean recovery obtained for MET and STG was 99.70 % and 99.40% respectively. The %RSD is less than 2, results were given in Table 3.

Precision

Results for repeatability expressed as %RSD, results were given in Table 3. The low values of %RSD indicate that the method is precise. Reproducibility was checked by analyzing the samples by another analyst using same instrument and same laboratory. There was no significant difference between the %RSD values, which indicates that the proposed method was reproducible, results were showed in Table 4.

Detection limit and quantification limit

LOD for MET and STG was 0.282 and 0.036 µg/ml respectively, while LOQ was 0.05 and 0.111 µg/ml respectively. Table no. 5.

Robustness

There was no significant change in the peak areas and retention times of MET and STG when the composition of mobile phase ±1 ml and flow rate was varied by ±0.2 ml. The results are showed in Table 6.

Specificity

No interference from any of the excipients was found at retention times of the examined drugs. In addition, the chromatogram of each drug in the sample solution was found identical to the chromatogram received by the standard solution at the wavelengths applied. These results demonstrate the absence of interference from other materials in the pharmaceutical formulations and therefore confirm the specificity of the proposed method. Table no.7.

System suitability

The acceptance criteria are % RSD of peak areas and retention time less than 2%, theoretical plates numbers (N) at least 2000 per each peak and tailing factors less than 2 for MET and STG and the results are shown in the Table 8.

DISCUSSION

In order to achieve simultaneous estimation of the two components, initial trials were performed with the objective of selecting adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and their proportions, detection wave length and concentrations of the standard solutions were carefully studied. Several solvents were tested in varying proportions. Finally, a mixture of Potassium Phosphate Buffer: ACN 55:45v/v was selected as the optimum mobile phase. The optimized chromatographic conditions were selected based on sensitivity, retention times and peak shape. The method was validated in terms of linearity, accuracy, precision, LOD, LOQ, robustness and specificity as per ICH guidelines. The accuracy data shows that the method is accurate within desired range. The LOD and LOQ values were low which indicates that the method is sensitive. The method was robust as minor changes in the chromatographic parameters did not bring about any significant changes in peak area and retention times of MET and STG.

CONCLUSION

The developed method for the simultaneous determination of MET and STG has advantage of sensitivity, accuracy, precision and low cost. The non-interference of tablet excipients make the method suitable for the simultaneous estimation of these drugs in tablets and hence can be used for routine quality control of MET and STG in pharmaceutical dosage form.

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Table 2: Linear regression data for the calibration curves

Metformin		Sitagliptin	
Concentration (µg/ml)	**Area**	**Concentration (µg/ml)**	**Area**
75	2589.996	7.5	188.517
100	3256.678	10.0	226.106
125	3757.966	12.5	249.907
150	4486.613	15.0	297.234
175	4889.965	17.5	334.666

Fig no.5 Linearity graph of Meformin

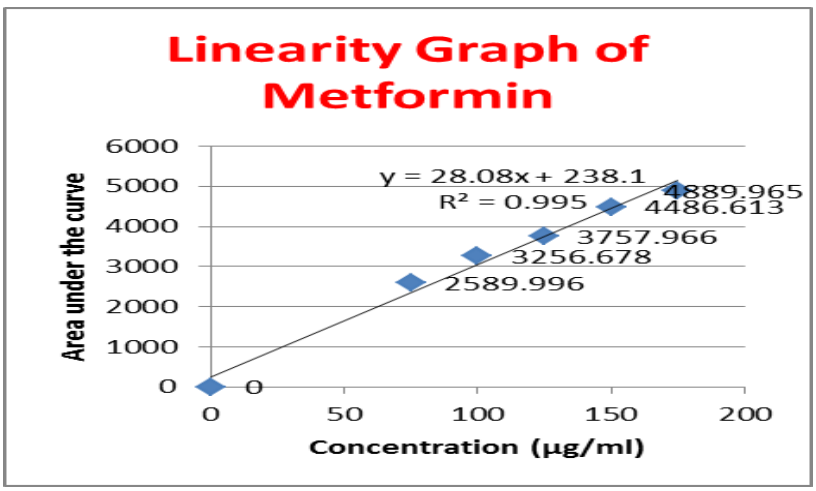

Fig no.6 Linearity graph of Sitagliptin

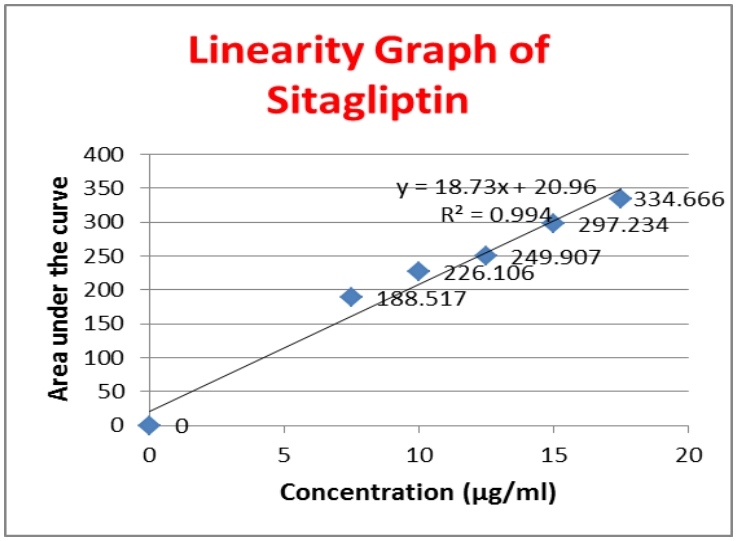

Table 3: Accuracy data for proposed method

Injection sample	Spike Level	Average Area	Amount add	Amount recovered	%Recovered	% of mean recovery
Metformin	105%-1	3478.217	104.59	104.12	99.55	99.70
	130%-1	3472.629	129.54	129.12	99.675	
	155%-1	3464.888	154.45	154.31	99.90	
Sitagliptin	10.5%-1	234.256	10.49	10.410	99.23	99.40
	13.5%-1	245.113	13.45	13.41	99.702	
	15.5%-3	300.586	15.41	15.30	99.28	

Table 4: Precision of the proposed HPLC method

S.No.	Metformin		Sitagliptin	
	Area	% Assay	Area	%Assay
1	3464.464	251.255	99.53	100.03
2	3451.364	250.721	99.52	100.02
3	3477.180	253.212	99.53	99.96
4	3466.574	249.136	99.44	99.95
5	2467.228	240.802	99.26	99.94
6	3442.539	251.082	99.28	99.99
Average	3294.892	249.368	99.426	99.98
STD DEV	40.5	4.393989	0.12611	0.0376
% RSD	1.231	1.7620	0.126	0.037

Table No.5 Detection limit and quantification limit results

DRUG NAME	LOD=3.3(SD/S).	LOQ = 10(SD/S).
Metformin	0.282	0.05
Sitagliptin	0.036	0.111

Table 6: Results of robustness for proposed method

Inj.Sample	Flow Rate(ml/min)	USP Plate Count	USP Tailing	Wavelength (nm)	USP Plate Count	USP Tailing
Metformin	0.8	2222	1.821	244	2045	1.783
	1.2	4431	1.783	248	4569	1.600
Sitagliptin	0.8	1938	1.789	244	2147	1.739
	1.2	4076	1.697	248	4377	1.474

Table no.7: Specificity results

Sample ID	Retention Time	Interference at	
		METFORMIN	SITAGLIPTIN
Blank	**No peaks observed at retention time of principle peaks.**	**Nil**	**Nil**
Placebo	**No peaks observed at retention time of principle peaks.**	**Nil**	**Nil**

Table 8: System suitability parameters

Parameters	Metformin	Sitagliptin
Tailing factor (T)	1.739	1.615
Number of theoretical plate(n)	2064	3901
Retention time (Rt)	2.123	4.953
Area	3472.629	245.113

Table no.9 ASSAY RESULT

Compound	Standard area	Sample area	Standard purity
METFORMIN	825.949	824.612	100.67
SITAGLIPTIN	284.554	287.747	99.54

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