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METHOD DEVELOPMENT AND VALIDATION OF METFORMIN HYDROCHLORIDE AND SAXAGLIPTIN BY RP-HPLC

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ABSTRACT

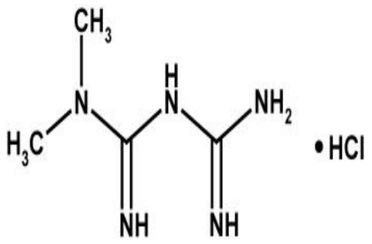
A new simple, rapid,economical reverse phase high performance liquid chromatographic method was developed for the determination of Metformin Hcl [MFH] and Saxagliptin [SGP] in bulk and dosage form. The separation was eluted on a Thermo Hypersilbds c_{18}(150mm x5 mm i.d. ,6μm) using a mobile phase mixture of potassium dihydrogen Phosphate buffer (0.05M) pH 6.8 and methanol in a ratio of 50:50 v/v at a flow rate of 1.0ml/min. Water is used asa diluent The detection was made at 294nm. The retention times were 1.4min for Metformin. Hydrochloride and 2.4min for Saxagliptin . Calibration curve was linear over the concentration range of 125-375μg/ml for Metformin.hydrochloride and 1.25 to 3.75 μg/ml for Saxagliptin mean recoveries obtained for metformin.hcl and saxagliptin were 99-100% and ,99-101% respectively.The propose method was validated as per the ICH guidelines parameters like Linearity, precision, accuracy, robustness limit of detection and limit of quantitation. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

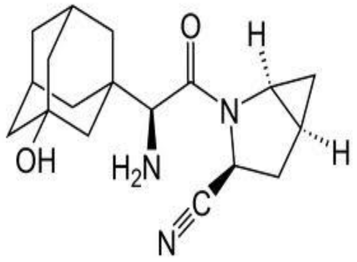
KEY WORDS: Saxagliptin, Metformin.Hydrochloride Tablets, C18 column, RP-HPLC.

1.INTRODUCTION

Metformin Hydrochloride(MTF) (C4H11N5.HCl) is 1, 1-dimethylbiguanidine monohydrochloride (Fig 1), is an anti-diabetic drug from the biguanide class of oral hypoglycaemic agents, given orally in the treatment of non –insulin-dependent diabetes mellitus[1] .Major action of metformin HCl in increasing glucose transport across the cell membrane in skeletal muscle[2-3.] But there is no officialmethod for the combination. Saxagliptin is a new oral hypoglycaemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs[4], licensed for the treatment of type II diabetes in combination with either metformin, a sulphonylurea or a thiazolidinedione. Saxagliptin once daily added to metformin therapy was generally well tolerated and led to statistically significant improvements in glycaemic indexes versus placebo added to metformin in patients with type 2 diabetes inadequately controlled with metformin alone[5-6].

Both drugs in combination of tablet dosage form in the ratio of 500:5 MFH: SGP. As per literature survey many methods have been reported the estimation of MFH and SGP individually or in combination with some other drugs[7-12]. With this present proposed method both MFH and SGP estimates simple and economical in tablet formulation. The chemical structures of the assayed compounds are given below.





2.MATERIALS AND METHODS

2.1 Chromatographic Conditions

Waters e 2695 separation module with high pressure liquid chromatographic instrument provided with thermo hypersil bds c_{18} (150mm x5 mm i.d. ,6µm) and 2489 PDA(photodioarray-detector), autoinjector, autosampler with Empower 2 software from Waters corporation, Milford USA was employed in the study. HPLC grade acetonitrile , water were purchased from E.Merck Co; Mumbai, India, and Potassium dihydrogen phosphate, ortho phosphoric acid AR grade were purchased from SD Fine Chem Mumbai, India were used in the study.

2.2. Standard Preparation

Accurately weighed about 500 mg of Metformine,5 mg of Saxagliptin working standards are transferred Separately into 100ml clean dry volumetric flask, added about 50ml of Water and sonicate 15 minutes to dissolve it completely and make the volume up to the mark with Water (Stock solution).

Further pipette out 5 ml of the Metformin hcl and 2.5 ml of Saxagliptin above stock solutions into a 100ml volumetric flask and diluted up to the mark with diluent to get the concentrations of 50µg/ml, 0.5µg/ml respectively. These stock solutions were filtered through 0.45µm membrane filter paper by using vacuum filter.

2.3.Sample Preparation

Twenty tablets were weighed and powdered. Average weight was calculated . Tranfered the 1182mg weight of sample in to 100ml of volumetric flask and add 50ml of Water and Sonicate 30 minutes and make up with Water. Transfer above solution in to 5 ml into 100 ml volumetric flask dilute to volume with water Then the solution was filtered through 0.45µ membrane filter.

METHODOLOGY

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for MFH and SGP was obtained with a mobile phase methanol:potassium dihydroen phasphae buffer ((0.05M)) with pH 6.8 (50:50 v/v) at a flow rate of 10ML /minto get better reproducibility and repeatability. Quantification was carried out at 294 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (fig.1). System suitability test parameters for MFH and SGP for the proposed method are reported in table1.

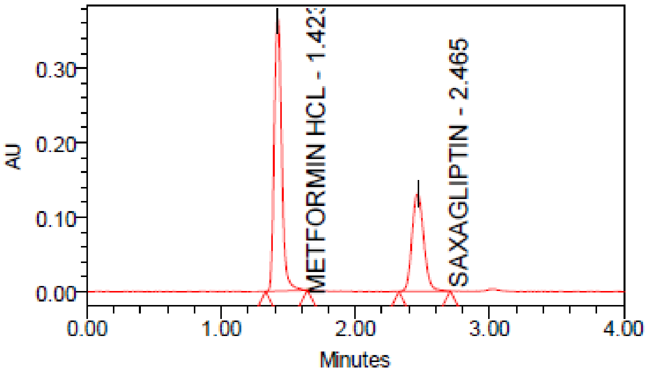


Fig: 1 Chromatogram of standard solution of MFH and SGP

Table 1: System Suitability Parameters.

System Suitability Parameters	Metformin.Hcl	Saxagliptin
Resolution	-	8.374
Tailing Factor	1.240	1.141
No.of theoretical Plates	3648	4295
Retention time	1.424	2.466
Similarity factor	1.00	1.00

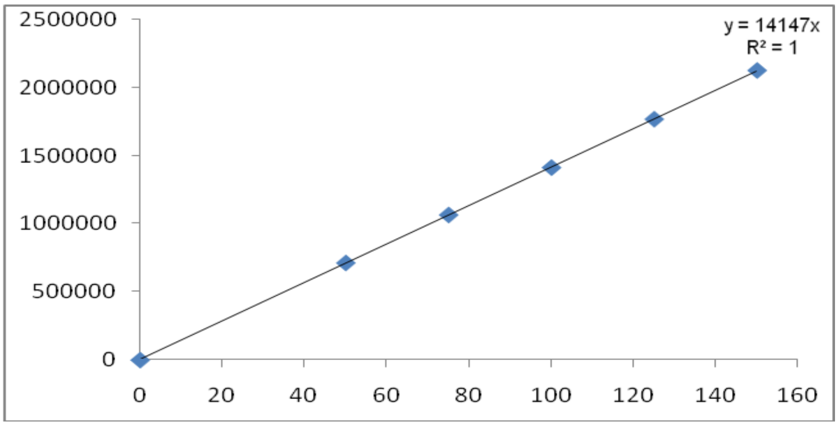


Fig .2 :Calibration curve for Metformin Hydrochloride

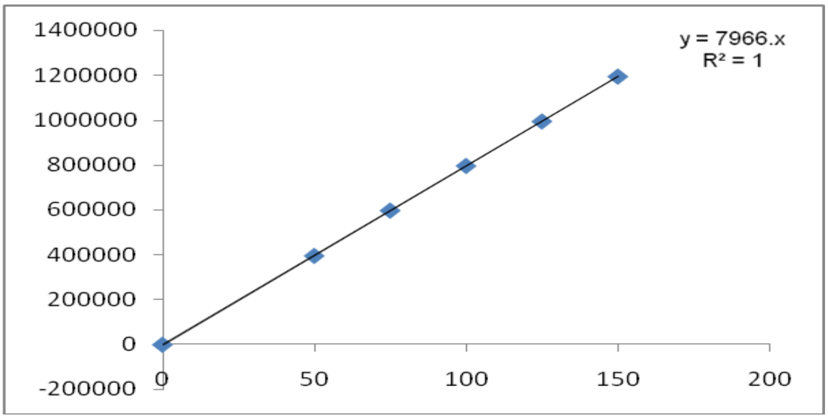


Fig.3 : Calibration curve for saxagliptin

Validation developed method

The proposed method has been validated for the simultaneous determination of MFH and SGP in tablet dosage form.[13] Calibration curves were constructed by plotting peak areas versus concentrations of MFH and SGP , and the regression equations were calculated. The calibration curves were plotted over the concentration range 125-375 µg/mL for MFH and 1.2-3.7 µg/ml for SGP. Aliquots (20 µl) of each solution were injected and analysed under the operating chromatographic conditions described as above. Regression parameters are mentioned in **Table 2**.

Table: 2. Summary For Validation Parameters

Parameters	Metformin.Hcl	Saxagliptin
Linearity range	125-375 µg/ml	1.2-3.7 µg/ml
Correlation Coefficient	0.99992	0.99997
Slope (m)	14128.76	7966
Intercept	2000	-728.071
Specificity	No Interference at Rt of the analyte peak	No Interference at Rt of the analyte peak
MethodPrecision (%Rsd)	0.1	0.0
Accuracy(%Rsd)	1.424	2.466
Robustness (%Rsd)	16.5	15.8
LOD	0.367µg/ml	0.004 µg/ml
LOQ	1.22 µg/ml	0.013 µg/ml

For the repeatability, the relative standard deviation (RSD) values for MFH and SGP were found to be 0.1 and 0.0%, respectively. The RSD values were found to be <2%, which indicates that the proposed method is repeatable. limit of detection (LOD) values for MFH and SGP were found to be 0.367 and 0.004 µg/ml, respectively, and limit of quantification (LOQ) values for MFHand SGPwere found to be 1.22 and 0.013 µg/ml, respectively (Table 2). These data show that the proposed method is sensitive for the determination of MFH and SGP.

Specificity of the method was confirmed from the resolution factor and peak purity data of the analyte. The resolution factor obtained for the MFH and SGP from the nearest resolving peak was >2 in all samples. The standard chromatogram of MFH and SGP display good resolute peak (Fig. 1)and no interference from excipients present in the formulation indicate specific nature of the method (Fig. 2).The peak purity data of the MFH and SGP show that no other excipient is coeluted with the drug and the peak of the drug is pure in nature. The recovery experiment was performed by the standard addition method. The mean recoveries obtained were 99.07-100%and 100% for MFH and SGP, respectively (Table 2). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 3.

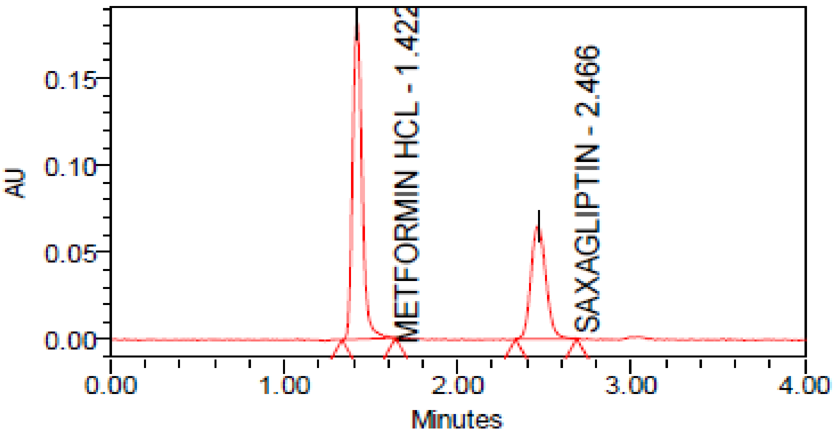


Fig: 4.

Fig. 4: Chromatogram of sample solution of MFHand SGP MFH (250µg/ml) is Metformin concentration and SGP (2.5 µg/ml) is Saxgliptin concentration in chromatogram of marketed formulation at 294 nm.

RECOVERY DATA

Table:3 Recovery data

S.No	Sample Name	Spike Level	Amount Added µg/ml	Amount Founded µg/ml	% Recovered	% mean
1	MFH	50 %	125.00	124.70	100 %	100%
		100%	250.00	248.68	99%	99%
		150%	374.365	373.11	100%	100%
2	SGP	50%	1.238	1.23	100%	100%
		100%	2.475	2.48	100%	100%
		150%	3.706	3.71	100%	100.3%

The robustness was studied by analysing the same samples of MFH and SGP by small but deliberate variation in the method parameters and the change in the responses of MFH and SGP were noted. Robustness of the method was studied by changing the flow rate by ±0.1 ml/min (flow rate 1.2 and 0.9 ml/min), column oven temperature by ±5° (25 and 35°) None of the alterations caused a significant change in resolution between MFH and SGP,peak area % RSD, retention time % RSD, tailing factor and theoretical plates and the results are reported in Table 4.

Table:4. Robustness Data for Met.HCL and Saxagliptin

S.No	Sample name	Metformin.Hcl				Saxagliptin				
		Rt	Area	tailing	plate count	Rt	Area	tailing	Plate count	Resolution
1	Temp-1	1.424	1394851	1.181	3305	2.466	795347	1.155	3841	7.947
2	Temp-2	1.420	1388675	1.175	3365	2.436	784487	1.140	3946	7.979
3	Flow-1	1.778	1738537	1.182	3596	3.080	970000	1.138	4286	8.363

4	Flow-2	1.187	1170393	1.190	2844	2.055	661655	1.178	3280	7.372
Mean			1423114				802872			
Std.de			234766				126845			
% Rsd			16.5				15.8			

Analysis of marketed formulation

The proposed validated method was successfully applied to determine MFH and SGP in their tablet dosage form. The amount found to be 201.1±2.62 and 501.45±2.61 mg/tablet for MFH and SGP, respectively. The result obtained for MFH and SGP was comparable with the corresponding labelled amounts The RP-HPLC chromatogram for MFH and SGP of sample was recorded and shown in **Fig. 2.**

RESULTS AND DISCUSSION

A RP-HPLC method was developed and validated for the determination of MFH and SGP in tablet dosage forms on a column thermo Hypersil bds c_{18} (150mm x5 mm i.d. ,6µm)with variable wavelength detection at 294 nm. The retention times of MFH and SGPwas 1.42 and 2.4min, respectively. Linear correlation was obtained between area and concentrations of MFH and SGP in the concentration ranges of 125-375 and 1.25-3.75 µg/ml, respectively. The low RSD values of intraday 0.1% for MFH and 0.0% for SGP at 294nm, reveal that the proposed method is precise. The LOD and the LOQ for MFH and SGP were found to be 0.367 and 0.004 µg/ml and 1.22 and 0.013 µg/ml, respectively. These data show that method is sensitive for the determination of MFH and SGP The recovery experiment was performed by the standard addition method. The mean recoveries were 99-101% and 100% for , respectively MFH and SGP(Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine MFH and SGP in their tablet dosage form. The results obtained for MFH and SGP were comparable with the corresponding labelled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence, the proposed method is applicable for the routine simultaneous estimation of MFH and SGP in pharmaceutical dosage forms. A simple, linear, accurate, specific and selective RP-HPLC method was developed and validated for estimation of MFH and SGP in their combined dosage form. In this proposed method the linearity is observed in the concentration range of 125-375µg/ml for MFH and 1.25-3.75 µg/ml for SGP with coefficient of correlation, (r^2)=0.9992and (r^2)=0.9997 for MFH and SGP, respectively at 294 nm. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the MFH and SGP in combined dosage form without any interference of excipients.

CONCLUSION

In the present investigation, we have developed a simple, sensitive, precise and accurate RP-HPLC method for the quantitative estimation of Metformin.Hcl and Saxagliptin in bulk drug and pharmaceutical formulations and a simple, sensitive, precise and accurate RP-HPLC method for the simultaneous estimation of Metformin.Hcl and Saxagliptin in bulk drug and pharmaceutical formulations. These methods can be used for the routine determination of Metformin.Hcl and Saxagliptin in bulk drug and in pharmaceutical formulations.

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