Research Article

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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN PHOSPHATE AND METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM

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Abstract

A simple, rapid, precise, accurate RP-HPLC method has been developed and validated for the simultaneous estimation of Sitagliptin and Metformin in combined dosage forms. Chromatographic separation was achieved with mobile phase consisting of Acetonitrile: Phosphate Buffer pH 3.0 in the ratio of 40:60 v/v with Supercil C18 (250 × 4.6 mm × 5 µm), column at a flow rate of 1 mL/min and detection wavelength was 236 nm. The retention times of Sitagliptin Phosphate and Metformin Hydrochloride was found to be 4.813 min and 2.390 min respectively. The method was validated in terms of Linearity, Range, Accuracy, Precision, Specificity, LOD, LOQ, Robustness and system suitability according to ICH guidelines. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis for determination of Sitagliptin Phosphate and Metformin Hydrochloride in tablet dosage form.

Keywords: Sitagliptin, Metformin, RP-HPLC, Development, Validation, Simultaneous estimation.

Introduction

Sitagliptin Phosphate (STG) is chemically 1,2,4-triazolo[4,3-a]pyrazine,7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl). It is used in the treatment of diabetes. It is an oral anti hyperglycemic (anti-diabetic) drug of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. Sitagliptin phosphate competitively inhibits dipeptidyl peptidase-4, an enzyme involved in the breakdown of incretins such as glucagon-like particle-1 (GLP-1) which potentiate insulin. Secretion in vivo. Inhibit ion of DPP-4 reduces the breakdown of GLP-1 and increases insulin secretion; this suppresses the release of glucagon from the pancreas and drives down blood sugar levels.[1]

Metformin Hydrochloride (MET) is chemically 3-(diaminomethylidene)-1,1 dimethylguanidine. It is an oral anti-diabetic drug which is the first line drug of choice for the treatment of type 2 diabetes, particularly in overweight or obese peoples and those with normal kidney function. Metformin improves hyperglycemia, primarily through its suppressive action on production of hepatic glucose (hepatic gluconeogenesis).[2]

A literature survey shows that a number of Derivative spectrophometry[3], ion pair chromatography[4], UV-spectroscopy[5], Spectro - flourimetri[6], Liquid chromatography tandem mass spectrometry[7,8,9], UPLC[10], HPLC[11,12,13,14] methods

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have been reported for the simultaneous estimation of STG and MET in pharmaceutical dosage forms in combination or combinations with other drugs.

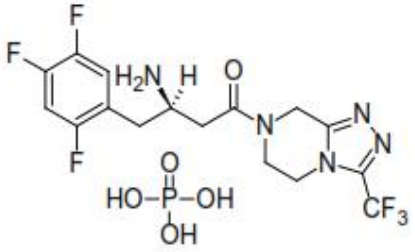

Fig. No. 01: Chemical structure of STG

So my aim to develop new method with optimum pH and less run time for simultaneous estimation of STG and MET in tablet dosage form.

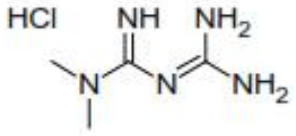

Fig. No. 02: Chemical structure of MET

Materials and methods

Chemicals and reagents

Drug samples were obtained from Getz Pharmaceuticals Research Pvt. Ltd., India. Tablets (Janumet, MSD Pharmaceuticals Pvt Ltd) Potassium Dihydrogen orthophosphate (Rankem /AR Grade) Dipotassium hydrogen orthophosphate (Rankem/AR Grade) Acetonitrile (Merck/HPLC Grade) Water (Merck/HPLC Grade) Methanol (Merck/HPLC Grade) O-phosphoric acid (Rankem/ Reagent Grade).

Instrumentation

Shimadzu HPLC system equipped with pump (LC-10ATVP), UV detector (SPD-10ADVP), column Supersil C18 (250×4.6 mm I.D) 5 µm particle size, Rheodyne injector fitted with 20µL loop and data read out by using spinchrom software.

Chromatographic conditions

Analysis was carried out at ambient temperature. Compounds were separated isocratically with a mobile phase consisting of Acetonitrile: phosphate buffer having pH 3.0 ± 0.2 in the ratio of 40:60 v/v at a flow rate of 1 mL/ min. The detection of the compounds was carried out at 236 nm. The mobile phase was filtered by using 0.45 µm membrane filter (Millipore, Bradford, MA), and sonicated in ultrasonicator for 15 minutes.

Preparation of phosphate buffer pH 3.0±0.2

Accurately weigh 1.625 gm of potassium dihydrogen orthophosphate and 0.39 gm of Dipotassium hydrogen orthophosphate in 550 mL of HPLC grade water and pH is adjusted to 3.0±0.2 with O-Phosphoric acid.

Preparation of standard stock solution

Stock solution was prepared by dissolving 125 mg of MET and 12.5 mg of STG in few mL of mobile phase in a 100 mLvolumetric flask. It sonicated and the volume was made to the mark with the mobile phase and filtered.

Working standard solution

From above stock solution 125µg/mL of MET and 12.5 µg/mL of STG working solution was prepared, pipette out 1 mL in 10 mL volumetric flask and the volume up to the mark was made with the mobile phase. The above solution is used for precision, specificity, robustness

Sample stock preparation

Twenty tablets were weighed accurately and powdered. A quantity of powder equivalent to 125 mg of MET and 12.5 mg of STG in 100 mL volumetric flask and make up mark with mobile phase. The above solution was sonicated and filtered.

Working sample solution

From above stock solution 125 µg/mL of MET and 12.5 µg/mL of STG working solution was prepared by pipette out 1 mL in 10mL volumetric flask and the volume up to the mark was made with the mobile phase. The above solution is used for assay.

Calibration and linearity

The calibration curves were constructed in the range of 75-175 µg/mL for MET and 7.5-17.5 µg/mL for STG. the solution were prepared by diluting 0.6, 0.8, 1.0, 1.2, 1.4 mL of standard stock solution to 10 mL with mobile phase.

Results and discussions

Optimization of chromatographic conditions by using different mobile phase compositions. The optimum chromatographic conditions found with acetonitrile and phosphate buffer pH 3 in the ratio of 40:60 v/v on Supercil column C18 (250×4.6 mm I.D) 5µm particle size with a flow rate of 1 mL/min and detection wavelength was selected as 236 nm. STG was eluted at 4.813 min with theoretical plates 4441, tailing factor 1.359 and resolution 9.428. MET was eluted at 2.390 min with theoretical plates 2000, tailing factor 1.912. So this condition was used for assay and the assay results were shown in Table 1.

Method validation

Method validation done according to the ICH guidelines for validation of analytical procedures.

System suitability

System suitability of method was carried out to verify that the resolution and reproducibility of the system are satisfactory for the analysis to be performed. Theoretical plates, tailing factor, Resolution parameters were determined and compared against the specifications and are presented in Table 2.

Precision

System Precision

System precision was carried out by using standard preparation for six times and the result was calculated. The %RSD was found to be less than 2%. This proves the method was precise. The result was shown in Table 3.

Method Precision

Method precision was carried out by using sample preparation for six times and the result was calculated. The %RSD was found to be less than 2%, which proves the method was precise. The result was shown in Table 4.

Linearity

The linearity of method was studied by preparing different concentration levels of standard solutions. The linearity range for MET and STG were found to be 75-175 µg/mL and 7.5-17.5 µg/mL, respectively. The regression equation for MET and STG were found to be $y = 30.92x – 985.1$ and $y = 9.889x – 32.36$ with coefficient of correlation, (R^2) 0.999 and 0.999, respectively. The linearity graph of MET and STG was shown in Fig 3 and Fig 4.

LOD and LOQ

LOD and LOQ is calculated from standard deviation of response from precision and slope from linearity

$$LOQ = 10\ \sigma / S$$

$$LOD = 3.3\ \sigma / S$$

Where,

σ is standard deviation from response

S is slope from calibration curve

The LOD for this method was found to be 0.072 µg/mL for MET and 0.0.307 µg/mL for STG respectively. The LOQ for this method was found to be 0.0.219 µg/mL for MET and 0.930 µg/mL for STG respectively.

Specificity

Specificity of the method was determined by comparing the retention times of MET and STG of standard solution with the retention times of MET and STG of sample solutions. Good correlation was obtained between the retention times of standard with sample shows that there is no interference of excipients from tablet dosage form. Specificity chromatograms given in Fig 5 and Fig 6.

Accuracy

The degree of accuracy of the method was determined by recovery studies in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard MET and STG were added to pre-analyzed samples and were analysed in proposed HPLC method. Results of recovery studies were shown in Table 5 and Table 6.

Robustness

Robustness of the method was performed by small deliberate variation in operating conditions like wave length (±2 nm) and flow rate (±0.1mL/min). The results were shown in Table 7.

From linearity the correlation coefficient R^2 values were found to be 0.999 for both drugs which shows that linear relationship between concentrations versus response. The proposed HPLC method was also validated for system suitability, system precision and method precision. The % RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 2000, which indicates efficient

performance of the column. The LOD of MET and STG were found to be 0.072 µg/mL and 0.307 µg/mL and LOQ of MET and STG were found to be 0.219 µg/mL and 0.930 µg/mL respectively, indicates the sensitivity of the method. The percentage of recovery of MET and STG were found to be 99.71 and 100.03 respectively shows that the proposed method is highly accurate.

Table No. 01: Results for assay

Drug	Label claim(mg)	Amount found(mg)	% Assay
STG	50	49.90	99.79
MET	500	498.17	99.63

Table No. 02: Results for system suitability parameters

Parameters	STG	MET
Retention times(min)	4.820	2.380
Theoretical plates	4533	2230
Tailing factor	1.220	1.92
Resolution	9.679	-

Table No. 03: Results for system precision

Injection	STG		MET	
	Retention times	Area	Retention times	Area
1	4.81	85.421	2.36	2811.265
2	4.81	85.452	2.36	2822.991
3	4.817	86.603	2.37	2810.69
4	4.813	87.213	2.37	2806.346
5	4.817	86.969	2.373	2807.444
6	4.783	86.926	2.337	2767.662
Average	4.808	86.43067	2.3617	2804.40
SD	0.013	0.794	0.0133	18.948
%RSD	0.27	0.92	0.56	0.68

Table No. 04: Results for method precision

Injection	STG		MET	
	Retention times	Area	Retention times	Area
1	4.82	85.431	2.38	2818.265
2	4.82	85.462	2.38	2822.991
3	4.827	86.613	2.38	2811.69
4	4.823	87.203	2.38	2806.346
5	4.817	86.969	2.373	2807.444
6	4.783	86.926	2.337	2769.662
Average	4.815	86.434	2.3717	2806.07
SD	0.016	0.788	0.0172	18.943
%RSD	0.33	0.91	0.73	0.68

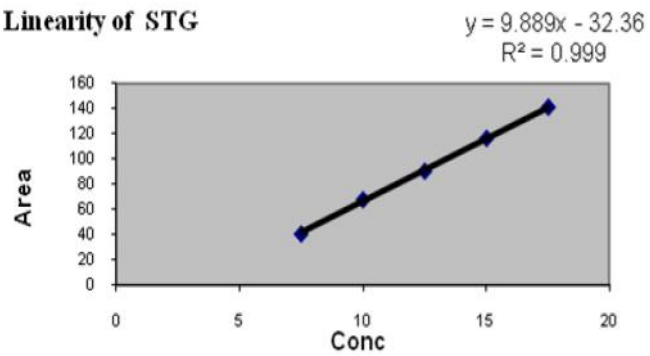

Fig. No. 03: Linearity graph of STG

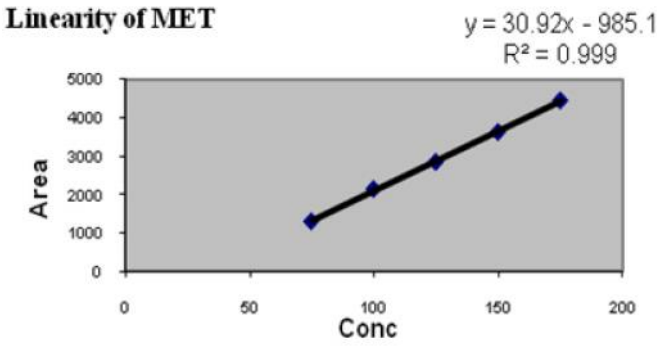

Fig. No. 04: Linearity graph of MET

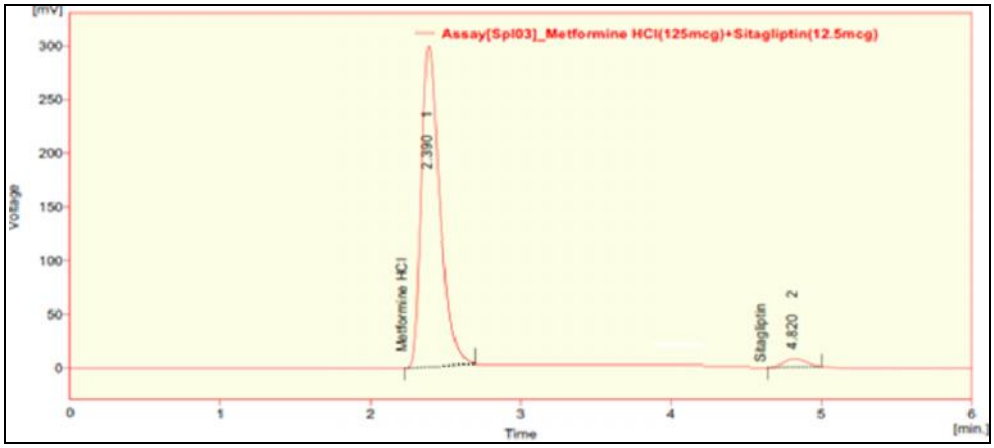

Fig. No. 05: Chromatogram of sample

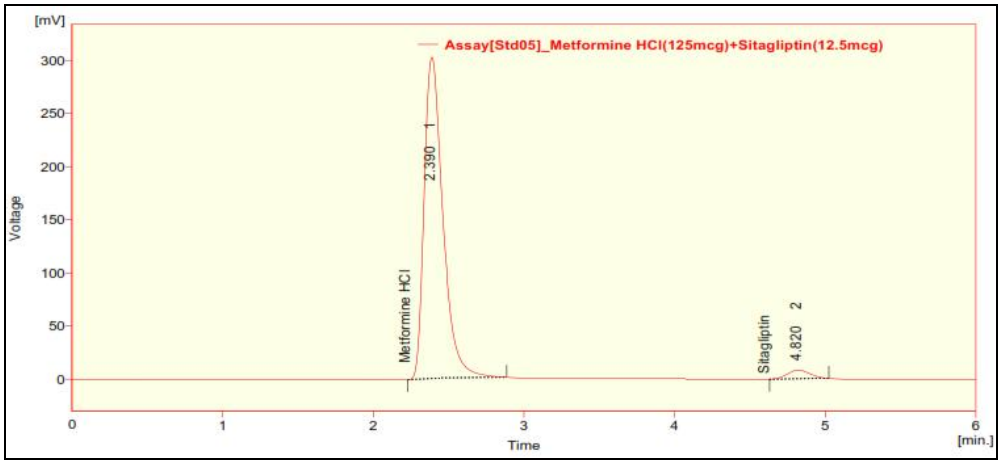

Fig. No. 06: Chromatogram of standard

Table No. 05: Results for Recovery of STG.

Conc	Amount present (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)*	Percentage Recovery *	% Mean Recovery
80%	10	0.5	9.89	98.91	100.03
100%	13	0.5	13.13	101.01	
120%	15	0.5	15.11	100.76	

***** Mean of three observations

Table No. 06: Results for Recovery of MET.

Conc	Amount present (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)*	Percentage Recovery *	% Mean Recovery
80%	100	0.5	105.16	100.15	99.71
100%	125	0.5	129.05	99.27	
120%	150	0.5	154.54	99.70	

***** Mean of three observations

Table No. 07: Results for Robustness

Chromatographic changes		Retention time(min)		Tailing factor	
		STG	MET	STG	MET
Flow rate (mL/min)	0.8	5.970	2.970	1.120	1.98
	1	4.817	2.373	1.317	1.98
	1.2	4.050	1.997	1.237	1.97
Wavelength (nm)	234	4.823	2.390	1.275	1.98
	236	4.817	2.373	1.317	1.98
	238	4.820	2.390	1.200	1.97

Conclusion

The develop RP-HPLC method was proves to be precise, rapid, accurate, linear, specific. It can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories in near future.

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