INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND SITAGLIPTIN PHOSPHATE IN BULK DRUG AND PHARMACEUTICAL FORMULATION

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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 Xterra Symmetry C-8 analytical column (100×4.6 mm I.D., 5 µm particle size). The optimum mobile phase consisted of methanol:acetonitrile:phosphate buffer in the ratio of 20:35:45 v/v/v (Phosphate buffer pH 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 254 nm. The retention times were 3.69 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 100-300 µg/ml for Metformin Hydrochloride and 10-30 µg/ml Sitagliptin Phosphate. Mean percent recovery of samples at each level for both drugs were found in the range of 101% for Metformin Hydrochloride and 102% 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-dimethylimidocarbonimidic diamide. 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-tetra hydro-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 insulin release[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 spectrophotometric[11,12] and HPLC[13] have been reported. Simultaneous determination of MET and STG in bulk and tablet dosage form were reported by using spectrophotometric[14], spectroflourometric [15] and HPLC[16] 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.

EXPERIMENTAL

Materials and reagents

Working standards of pharmaceutical grade MET (Batch No.: 3489/201), STG (Batch No.: 5436/501) were obtained as generous gifts from Merck Sharp Dohme, USA. They were used without further purification and certified to contain 99.96% and 99.99% (w/w) on dry weight basis for MET and STG respectively. Fixed dose combination tablets (Brand Name: Janumet) containing 500 mg of MET and 50 mg of STG and procured from Merck Sharp Dohme, India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

Chromatographic system and conditions

Separation was performed with Waters HPLC equipped with a pump-515, auto sampler-2707 and UV detector-2998, operated at 261 nm. Empower software was applied for data collecting and processing. A Systronics-361 pH meter was used for pH measurements. The separation was achieved on a Xterra C-8 (100 x 4.6 mm, 5 µ) analytical column. The mobile phase consisted of methanol : acetonitrile: phosphate buffer in the ratio of 20:35:45 v/v/v (pH 8 was adjusted with sodium hydroxide). The flow rate was 1.0 ml/min and UV detection was performed at 261 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45 µ membrane filter (Millipore, Ireland). The injection volume was 20 µl and all the experiments were performed at ambient temperature.

Preparation of standard stock solution

Accurately weigh and transfer 100 mg of MET and 10 mg of STG 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 solution

Twenty tablets were accurately weighed and crushed in to a fine powder. An amount of powder equivalent to 50 mg of MET and 5 mg of STG transferred in to 10 ml volumetric flask and 10 ml of mobile phase was added to it. The mixture was sonicated to dissolve and then made volume up to the mark with mobile phase and the solution was filtered through 0.45 µm filter paper. From the above stock solution pipette out 1ml of the solution in to a 10 ml volumetric flask made up to volume with mobile phase to yield concentration of MET (500 µg/ml) and STG (50 µg/ml). A 20 µl sample was injected six times under optimized chromatographic conditions. The peak areas were measured at 261 nm.

Method validation

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

Six different concentrations of the mixed standard drugs of MET and STG were prepared for linearity studies and injected into system (n=6). 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 (50, 100, and 150%) of the labeled claim to the excipients. At each level, six 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), inter-mediate 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 (300 µg/ml) and STG (30 µg/ml).

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.

System suitability

The system suitability was evaluated by six replicate analysis of MET and STG mixture at concentration of 300 µg/ml and 30 µg/ml. The acceptance criteria are % RSD of peak areas and retention time less than 2%, theoretical plates numbers (N) at least 4500 per each peak and tailing factors less than 1.5 for MET and STG.

RESULTS

A typical chromatogram recorded at 261 nm is shown in Figure 3. The retention times of MET 3.69 min. and STG 4.90 min. respectively. The analyte peaks were well resolved.

Method validation

Linearity

The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 100-500 µg/ml and 10-50 µg/ml for MET and STG respectively. Linear regression data for the calibration curves are given in Table 1.

Accuracy

The % mean recovery obtained for MET and STG was 101% and 102% respectively. The %RSD is less than 2, results were given in Table 2.

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 3.

Detection limit and quantification limit

LOD for MET and STG was 0.42 and 0.24 µg/ml respectively, while LOQ was 1.41 and 1.8 µg/ml respectively.

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 ±0.1 ml. The results are showed in Table 4.

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.

System suitability

The acceptance criteria are % RSD of peak areas and retention time less than 2%, theoretical plates numbers (N) at least 4500 per each peak and tailing factors less than

1.5 for MET and STG and the results are shown in the Table 5.

Quantification of MET and STG in tablet dosage form

The proposed method was applied to the simultaneous determination of MET and STG in tablets. The results of the assay yielded 100.14±0.33% for MET and 99.84±0.24% for STG, of label claim of the tablets. The assay results showed that the method was selective for the simultaneous determination of MET and STG without interference from the excipients used in the tablet dosage form. The results are shown in the Table 6.

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 methanol: acetonitrile: buffer (20:35:45 v/v/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.

ACKNOWLEDGEMENTS

The author wishes to thanks Merck Sharp Dohme, USA for providing pure metformin hydrochloride and sitagliptin phosphate as gift samples.

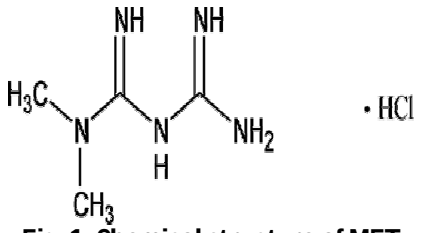

Fig. 1: Chemical structure of MET

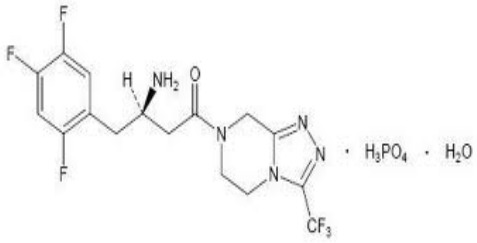

Fig. 2: Chemical structure of STG

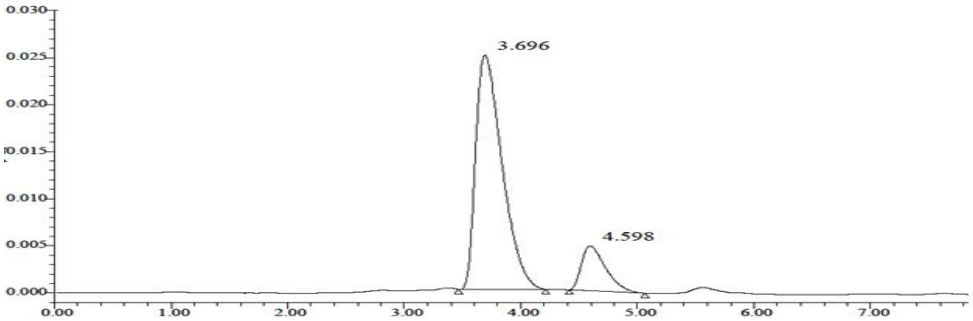

Fig. 3: Typical chromatogram of MET and STG in pharmaceutical formulation

Table 1: Linear regression data for the calibration curves[a]

Concentration of MET (µg/ml)	Concentration of STG (µg/ml)	Mean peak area of MET	Mean peak area of STG
100	10	270286	27012
200	20	363241	32133
300	30	443366	36801
400	40	530842	40954
500	50	606245	45342

[a]n=6

Table 2: Accuracy data for proposed method[a]

Spiked level of drug (%)	Amount of drug added (µg/ml)		Amount of drug found (µg/ml)		% Recovery	
	MET	STG	MET	STG	MET	STG
50	15	150	15.29	150.22	105.0	104.0
100	30	300	30.2	300.2	102.0	102.0
150	45	450	45.7	450.3	98.0	102.0

[a]n=6

Table 3: Precision of the proposed HPLC method

Conc. of MET (300 µg/ml) and STG (30 µg/ml)	Peak area of MET		Peak area of STG	
	Intra-day	Inter-day	Intra-day	Inter-day
Injection-1	397081	404582	33280	33402
Injection-2	407680	401584	34312	33481
Injection-3	408278	402145	34251	34170
Injection-4	410485	405102	34550	33741
Injection-5	412063	416542	34601	34870
Average	407117	405991	34419	33393.2
Standard Deviation	5877.7	6089.18	149.7	603.4
% RSD	1.44	1.49	0.43	1.7

Table 4: Results of robustness for proposed method[a]

Factor	Level	Retention time		Asymmetry	
		MET	STG	MET	STG
A: Flow rate (ml/min)					
0.9	-1	3.70	4.60	1.24	1.34
1.0	0	3.69	4.59	1.20	1.32
1.1	+1	3.65	4.54	1.19	1.28
%RSD		0.7	0.7	0.21	0.25
B: % of methanol (ml)					
19	-1	3.72	4.61	1.21	1.34
20	0	3.69	4.59	1.20	1.32
21	+1	3.58	4.58	1.18	1.28
%RSD		0.14	0.12	0.4	0.7

[a]n=6

Table 5: System suitability parameters

Parameters	MET	STG
Linearity (µg/ml)	10-500	10-50
Correlation coefficient	0.998	0.998
Theoretical plates	5401	4547
Tailing factor	1.20	1.32
LOD (µg/ml)	0.42	0.24
LOQ (µg/ml)	1.41	0.8

Table 6: Results of sample analysis for proposed method[a]

Brand name	Analyte	Label claim per tablet (mg)	% Analyte estimated (mean±SD)	%RSD
Janumet	Metformin	500	100.1±0.33	0.3302
	Sitagliptin	50	99.84±0.24	0.2395

[a]n=6

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