Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com (ISSN : 2277 -4572)

Research Article

A VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND SITAGLIPTIN IN ITS BULK AND PHARMACEUTICAL DOSAGE FORMS

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DOI: 10.7897/2277-4572.06671

Received on: 04/12/17 Revised on: 24/12/17 Accepted on: 18/01/18

ABSTRACT

The aim of the study is to develop and validate a simple, accurate, precise and rapid isocratic reverse-phase high-performance liquid chromatographic method for simultaneous determination of Metformin and Sitagliptin in bulk and tablet formulations. Chromatographic separation was supported on Symmetry C18 column (4.6×150mm) 5µ column with a blend of methanol**:** phosphate buffer (70:30) as mobile phase at a flow rate of 1 ml/min. UV detection was performed at 271 nm using HPLC Waters Separation module 2695, with Empower software version-2. The retention times were found to be 1.694minutes and 3.334 minutes for Metformin and sitagliptin respectively. Calibration plots were linear for both the drugs (r^2=0.999) over the concentration range 50-250 µg/ml for Metformin 5-50 µg/ml for sitagliptin. The LOD and LOQ values for Metformin and Sitagliptin were found to be 2.17 µg/ml, 0.0372µg/ml and 6.60 µg/ml and 0.112 µg/ml respectively. The optimized method was validated in accordance with ICH guidelines(IC(R1)) for accuracy, precision, specificity, linearity, and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. As the method shows high recovery and low relative standard deviation which confirms the suitability of the method for routine determination of Metformin and Sitagliptin in bulk drug and tablet dosage forms.

Key words: Metformin, Sitagliptin, simultaneous, RP-HPLC method and validation, ICH.

INTRODUCTION

Metformin is chemically 1-carbamimidamido-N,N-dimethylmethanimidamide with molecular formula $C_4H_{11}N_5$ and molecular weight of 129.16 gm/mol with an anti- hypoglycemic activity. It is freely soluble in water, insoluble in acetone, ether and chloroform. Sitagliptin is chemically (3R)-3-amino-1-[3-(trifluoromethyl)-5H,6H,7H, 8H[1,2,4]triazolo[4,3- a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one. Sitagliptin is a compound with molecular formula $C_{16}H_{15}F_6N_5O$ molecular weight of 407.314 gm/mol and used as an antidiabetic drug[2]. Sitagliptin is slightly soluble in methanol, very slightly soluble in ethanol, acetone and acetonitrile, insoluble in isopropanol and isopropyl acetate. The literature survey indicates that metformin and sitagliptin estimation were carried by various UV, HPLC and HPTLC analytical techniques[3-14]. As there are no suitable simple RP- HPLC methods for simultaneous estimation of metformin and sitagliptin were reported. Hence, this study was performed to develop a specific method for estimation of metformin and sitagliptin simultaneously using RP-HPLC.

MATERIALS AND METHODS

All the chemicals and reagents procured and used were of AR/HPLC grade. Pure standards of metformin and sitagliptin gift samples obtained from Merck India. HPLC grade methanol, acetonitrile as well as AR grade ortho phosphoric acid were obtained from Merck India (Mumbai, India). Chromatographic analysis was done using Waters HPLC Separation module 2695 using UV detector 2487 with Empower-software version-2.

Preparation of mobile phase

Isocratic mixture of 70 volumes of pH 3 phosphate buffer (70%) and 30 volumes of methanol (30%) were taken and degassed for 5 minutes in ultra sonicator and vaccum filtered through 0.45 µ filter.

Preparation of standard stock solution of Metformin

10 mg of metformin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of standard stock solution of Grazoprevir

1 mg of sitagliptin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of tablet sample solution

Accurately 10 tablets (each tablet contains metformin 500mg and sitagliptin 50mg) were weighed and transferred into a clean dry mortar and crushed with clean pestle to get uniformity. The amount equivalent to 10 mg of metformin and

1mg sitagliptin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Procedure

10µl of the prepared standard and sample solutions were injected into the chromatographic system and the areas of metformin and sitagliptin peaks were measured.

ANALYTICAL METHOD VALIDATION[15-17]

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analysed sample solution at three different levels 100%, 120%, 140%. Each concentration was measured in triplicate and the percentage recovery and percentage mean recovery were calculated for both the drugs as depicted in Table 1.

Precision (Repeatability)

Procedure

The standard solutions of metformin and sitagliptin were injected repeatedly for six times and the areas for all six injections in HPLC were measured and %RSD was calculated and depicted in Table 2.

Specificity

Specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

LOD and LOQ

LOD and LOQ values were calculated by diluting known concentrations of metformin and sitagliptn till the normal response was approximately 3 to 10 times the standard deviation (SD) of response (peak area) for the three replicate determinations.

Linearity

Linearity was calculated by preparing aliquots of standards to obtain final concentrations of 50-250 µg/ml for Metformin 5-50 µg/ml for sitagliptin. Around 10 µl of the standard solutions were injecting into the column and peak areas were recorded. Calibration plots were constructed with average peak areas versus concentrations and regression equations were figured for both the drugs.

Robustness

To demonstrate the robustness of the method, both the drug solutions were injected at variable conditions like varied flow rate and mobile phase composition.

a) Standard solution of metformin and sitagliptin were analyzed at varied flow rates 0.8ml/min and 1.2 ml/min.

b) Standard solution of metformin and sitagliptin were analysed at varied mobile phase compositions from more organic phase to less organic phase

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

RESULTS AND DISCUSSION

The objective was to develop a unique HPLC method for the simultaneous detection and quantitation of metformin and sitagliptin. The isobestic point was measured by preparing 10µg/ml of individual and mixed standards and the solution was scanned in U.V region from 200-400nm. The intersection spectrum of metformin and sitagliptin was obtained and the isobestic point was observed at 271 nm. Many trails were performed with various column and several mobile phases compositions under gradient and isocratic conditions to optimize appropriate conditions for the detection and quantification of metformin and sitagliptin.

Although the use of buffers is advocated and often used in RP chromatographic separations, it would be prudent to avoid buffers in view of the harsh pH conditions and inevitable accumulation of salt in the HPLC system, column and column frits. Hence it is most desirable that a simple HPLC mobile phase and pH conditions that provide satisfactory resolution of sample components for the purposes of estimation at lowest possible concentrations. This study results indicate a good resolution between the compounds with appropriate peak shapes and retention times were achieved on a SYMMETRY C18 column (4.6×150mm) 5µ column with a blend of methanol: phosphate buffer (70:30) as mobile phase at a flow rate of 1 ml/min under ambient column temperature.

A demonstrative chromatogram showing resolution between metformin and sitagliptin is shown in Fig. 1.

Metformin and sitagliptin were eluted at 1.694mins and 3.334 mins respectively and the percentage purities were 100.27% and 99.87% respectively. Theoretical plates for metformin and sitagliptin were 2294 and 4891 whereas tailing factor was 1.27 and 1.03 respectively with a resolution of 8.67. The developed method was validated as per ICH, Q2 (R1) guidelines.

The linearity of metformin and sitagliptin were obtained in the concentration range of 50µg-250µg and 5µg-50µg with correlation coefficient (r2) of 0.999. Metformin and sitagliptin shows LOD of 2.17 and 0.0372, and LOQ of 6.60and 0.112 respectively with % recovery of 99.56% and 99.48% along with% RSD of 0.27 and 0.40 as summarized in Table 1. The % RSD for intermediate precision was 0.27 and 0.94 respectively. The precision study was precise, robust, and repeatable and the Results for Method precision are depicted in Table 2. The robustness evaluation for both the drugs are presented in Table 3. Insignificant differences in peak areas and less variability in retention times were observed. Overall summarized method validation results and system suitability test parameters are summarized in Table 4.

Table 1: Recovery results for Metformin and Sitagliptin

Recovery level	Amount taken		Amount recovered	
	Metformin Taken(mcg/ml)	Sitagliptin Taken(mcg/ml)	Metformin (mcg/ml)	Sitagliptin (mcg/ml)
50%	5	0.5	4.96	0.49
	5	0.5		
	5	0.5		
100%	10	1	9.98	1.05
	10	1		
	10	1		
150%	15	1.5	15.02	1.49
	15	1.5		
	15	1.5		
Average % Recovery			99.56%	99.47%

Table 2: Results for Method precision of Metformin and Sitagliptin

Metformin				**Sitagliptin**		
Injection Number	Retention Time	Area		Injection Number	Retention Time	Area
1	1.688	1817589		1	3.293	376633
2	1.690	1834970		2	3.257	380765
3	1.689	1840643		3	3.270	382506
4	1.685	1836875		4	3.263	378768
5	1.688	1825778		5	3.293	387899
6	1.687	1835446		6	3.267	379789
avg	1.688	1831067		avg	3.277	379967.9
stdev		12012.5		stdev		3016.1
%RSD		0.7		**%RSD**		0.8

Table 3: Robustness of Metformin and Sitagliptin

Parameter	**METFORMIN**		**SITAGLIPTIN**	
	USP Plate Count	**USP Plate Count**	**USP Tailing**	**USP Tailing**
Flow rate 0.8 ml/min	2590	5435	1.04	1.39
Flow rate 1 ml/min	2294	4891	1.03	1.27
Flow rate 1.2 ml/min	2146	4781	1.04	1.26
Organic Phase 5 % less	2347	1.44	5437	0.99
Organic Phase *Actual	2294	1.27	4891	1.03
Organic Phase 5 % more	2239	1.13	4817	1.05

Table 4: Summary of validation and system suitability parameters

Parameter (Units)	Metformin	Sitagliptin
Linearity range (µg/ml)	50-250 µg/ml	5-50 µg/ml
Correlation coefficient	0.999	0.999
LOD (µg/ml)	2.17µg/ml	0.0372 µg/ml
LOQ (µg/ml)	6.60µg/ml	0.112 µg/ml
Recovery (%)	99.56%	99.48%
Retention time±allowable time (min.)	1.69±0.20	3.33±0.20
% Purity	100.3%	99.9%
Theoretical Plate	2294	4891
Tailing Factor (asymmetry factor)	1.27	1.03

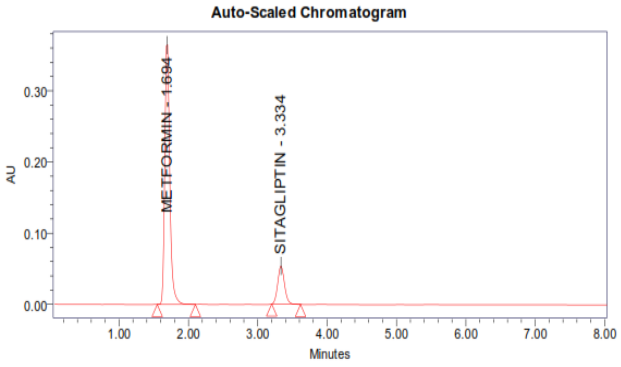

Fig. 1. Chromatogram of Metformin and Sitagliptin using methanol: phosphate buffer (70: 30v/v)

A validated HPLC method for the simultaneous quantification of metformin and sitagliptin has been established as per ICH guidelines. It has shown that the developed method achieved accuracy, reproducibility, repeatability, linearity, precision, and selectivity, which prove the reliability of the method. The method enabled accurate, sensitive, and reproducible quantification of tablet formulation in routine analysis. The result shows that the method could find practical application as a quality control tool for the simultaneous estimation of two drugs from their combined dosage form in a quality control laboratory.

CONCLUSION

The proposed method is simple, accurate, precise and selective for simultaneous estimation of metformin and sitagliptin in pure and in tablet dosage forms. The method is economical, rapid and not required any sophisticated instruments contrast to chromatographic method. Hence it can be effectively applied for the routine analysis of metformin and sitagliptin in pure and in tablet dosage forms.

ACKNOWLEDGEMENTS

The authors thank Mr Venu Gopal Reddy, Chairman, Bharat Institutions, Mangalpally for providing the required facilities and constant encouragement to carry out this research work.

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How to cite this article:
Sumalatha Nallagundla *et al*. A validated RP-HPLC method for the simultaneous estimation of Metformin and Sitagliptin in its bulk and pharmaceutical dosage forms. J Pharm Sci Innov. 2017; 6(6):131-134.
http://dx.doi.org/10.7897/2277-4572.06671

Source of support: Nil, Conflict of interest: None Declared