Research Article **www.ijcps.com**

International Journal of Chemical and Pharmaceutical Sciences
2017, Mar., Vol. 8 (1)

ISSN: 0976-9390
IJCPS

Simultaneous estimation of Metformin HCl and Sitagliptin in drug substance and drug products by RP-HPLC method

Butti Aruna*, Senthil Kumar B and Parthiban P.

Nova College of Pharmaceutical Education & Research, Jupudi, Vijayawada, Andhra Pradesh, India.

* **Corresponding Author:** E-Mail: arunabutti191@gmail.com

Received: 10th Mar 2017, Revised and Accepted: 18th Mar 2017

ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Metformin and Sitagliptin in drug substances and drug products Isocratic elution at a flow rate of 1 mL/min was employed on a Luna C18 (250 mm x 4.6mm,I.D., 5µm particle size) at ambient temperature. The mobile phase consisted of Buffer (0.1% OPA): ACN (50:50) (v/v). The detection wavelength was 285nm and 20µl sample was injected. The Metformin and Sitagliptin eluted at the retention time of 3.974 and 5.721 respectively. The method was validated as per the ICH guidelines. The developed and validated method was successfully applied for routine analysis of Metformin and Sitagliptin in drug substances and drug products.

Keywords: Metformin, Sitagliptin, RP-HPLC, Method development and validation.

1. INTRODUCTION

HPLC is one among the most useful tools, available for quantitative analysis. High Performance Liquid Chromatography is a special branch of column chromatography, in which the mobile phase ids force through the column at high speed. As a result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially. The main components of high performance liquid chromatography are shown in the following schematic diagram (Figure 1) [1-5].

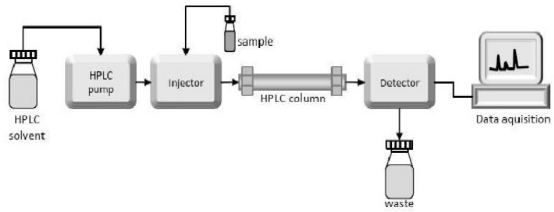

Figure -1: Schematic diagram of HPLC.

Sitagliptin (Figure 2) (INN, previously identified as MK-0431 and marketed as the phosphate salt under the trade name Januvia) is an oral antihyperglycemic (antidiabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. It was developed, and is marketed, by Merck & Co. This enzyme- inhibiting drug is used either alone or in combination with other oral antihyperglycemic agents (such as metformin or a thiazolidinedione) for treatment of diabetes mellitus type 2 [6-9].

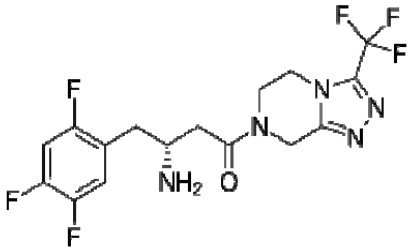

Figure -2: Structure of Sitagliptin.

Metformin (Figure 3), marketed under the trade name Glucophage among others, is the firstline medication for the treatment of type 2 diabetes. This is particularly true in people who are overweight. It is also used in the treatment of polycystic ovary syndrome. Limited evidence suggests Metformin may prevent the cardiovascular disease and cancer complications of diabetes. It is not associated with weight gain. It is taken by mouth [6-9].

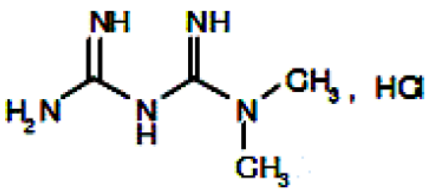

Figure – 3: Structure of Metformin HCl.

2. MATERIALS AND METHODS

2.1. Chemicals and solvents.

HPLC grade Water, Ortho Phospharic Acid, Acetonitrile was used in this experiment.

2.2. Instruments

HPLC Waters 2695 assisted with Empower software (Alliance). Ultrasonicator (Unic hrome),. Weighing balance (Denver) was used.

2.3. Methodology

2.3.1. Preparation of Buffer

1 mL Orthophospharic acid was diluted dissolved in 1000 mL of Water. Filtered through 0.45 µ membrane filter.

2.3.2. Preparation of mobile phase

Mixed buffer and Acetonitrile in the ratio of 50: 50 (% v/v)

2.3.3. Preparation of diluents

Used mobile phase as diluents.

2.3.4. Preparation of Standard Solution

Weighed accurately about 100.1 mg of Metformin working standard and 10.2 mg of Sitagliptin working standard into a 100 mL volumetric flask. Added 80 mL of diluent, sonicated to dissolve and diluted up to the mark with diluent. Further diluted 5mL of the above solution to 50 mL with the diluent. Filtered through 0.45 µ Nylon syringe filter.

2.3.5. Preparation of Sample solution

Weighed 10 tablets and calculated the average weight, then crushed the tablets in to fine powder. Weighed accurately about 136.06 mg of powdered sample taken into a 100 mL volumetric flask. Added 70 mL of diluent, sonicated to dissolve and diluted up to the mark with diluent. Further diluted 5 mL to 50 mL with the diluent. Filtered through 0.45µ Nylon syringe filter.

2.3.6. Determination of working wavelength (λ max)

The wavelength of maximum absorption of the solution of the drug in acetonitrile were scanned using Photodiode spectrophotometer within the wavelength region of 200–400 nm against acetonitrile as blank. The spectra of drug shows at 285 nm (Figure 4), Thus 285 nm was selected as detector wavelength for the HPLC chromatographic method.

2.3.7. Optimized Chromatographic conditions

Column: Luna C18 250mmx4.6mm, 5µm

Elution mode: Isocratic

Mobile phase: Buffer: ACN (50: 50) (% v/v)

Flow rate: 1.0 mL /min

Detection wavelength: 285 nm

Injection volume: 20 µL

Column oven temperature: 25°C

Run time: 10 min

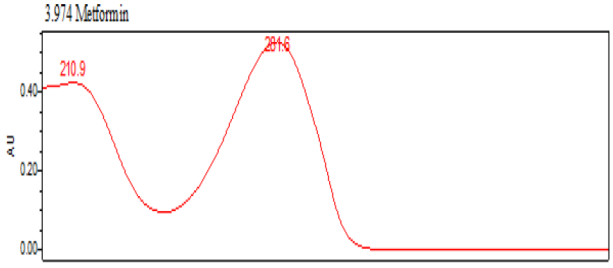

Figure – 4: Selection of Wavelength.

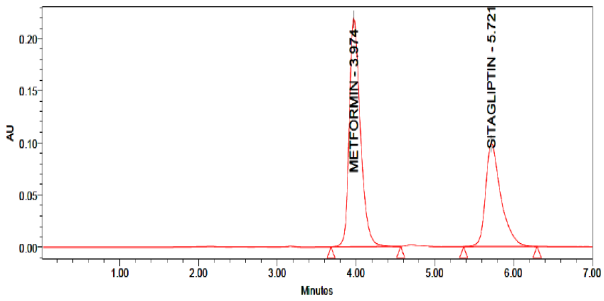

Figure - 5: Chrmatogram of Metformin and Sitagliptin.

2.3.8. Method validation

The developed and optimized method was validated as per the ICH Q2(R1) guidelines.

3. RESULTS AND DISCUSSION

3.1. Optimization of chromatographic condition

During the selection of chromatographic conditions numbers of trails were carried out and the best trail was selected for optimized method.

3.2. Method validation

The validation of HPLC method for the determination of Metformin and Sitagliptin as per the protocol and to demonstrate that the method is appropriate for its intended use was studied for the following parameters. All the validation parameters were carried out according to ICH.

3.3. System suitability

The system suitability of developed method was conducted through the validation studies by using 100.1+10.2 µg/mL Metformin and Sitagliptin. System suitability prior to analysis was investigated by checking parameters like tailing factor, retention times and number of

theoretical plates. The results were found to be within the limits.

Acceptance Criteria:

- The no. of Theoretical plates should not be less than 3000.
- The Tailing factor should not be more than 2.0.
- The % RSD should not more than 2.0

3.3. Linearity and Range

Linearity of an analytical method is its ability to elicit the test results that are directly, or by well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. Linear correlation was obtained between peak area Vs concentration of Metformin and Sitagliptin were in the range of 10.01-150.15 µg/mL and 1.06 - 15.02 µg/mL. The linearity of the calibration curve was validated by the high value of correlation co-efficient of regression equation. The Range of an analytical method is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated with precision, accuracy and linearity.

Acceptance Criteria:

Correlation coefficient should be not less than 0.999.

3.4. Accuracy

The accuracy experiments were carried out by the standard addition method at 50%, 100% and 150% levels and the recoveries obtained were 99.95 to 100.55% for both Metformin and Sitagliptin.

Acceptance Criteria:

The mean % recovery at each level should not be less than 98% - 102%.

3.5. Precision

Method repeatability:

The precision of the instruments was checked by repeatedly injecting (n=6) solutions of 100.01µg/mL Metformin and 10.2 µg/mL Sitagliptin.

Intermediate Precision (Reproducibility):

The intra-day and inter-day precision of the proposed methods were determined by the corresponding responses three times on the same day and on three different days over a period of one week for three different concentration of 100.01 µg/mL Metformin and 10.2µg/mL Sitagliptin. The low % RSD values of for Metformin and Sitagliptin were reveal that the proposed method was precise.

Acceptance Criteria:

The % RSD for the absorbance of six replicate injections results should not be more than 2%.

3.6. Robustness and Ruggedness

Robustness of the method was determined by carrying out the analysis at two different mobile phase (i.e. 40±5) and two different flow rates (i.e. 1±0.2 mL/min) The high % RSD values of robustness and for Metformin and Sitagliptin with change in flow rate indicates that the method is not robust for change in flow rate. The low % RSD values of robustness and for Metformin and Sitagliptin with change in Organic phase that the proposed method is robust.

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective peak areas were noted. The result was indicated by % RSD.

3.7. Solution stability stability study

Standard and sample solution was subjected of solution stability studies.

3.8. Degradation studies

3.8.1. Acid Degradation (5N HCl)

Diluted sample in a 100ml volumetric flask, add 50ml of diluent. Add 3ml of 5N HCl and heated at 70°C for 30 mins on a water bath. Remove the flask from the water bath and allow the flask to cool at room temperature. Add 3 ml of 5N NaOH to neutralize the solution. Cooled to room temperature and diluted to volume with diluent and mixed.

3.8.2. Base Degradation (5N NaOH)

Diluted sample in a 100ml volumetric flask, add 50ml of diluent add 3ml of 5N NaOH and heated at 70°C for 30 mins on a water bath. Removed the flask from the water bath and allow the flask to cool at room temperature. Add 3ml of 5N HCl to neutralize the solution. Cooled to room temperature and diluted to volume with diluent and mixed.

3.8.3. Peroxide Degradation (30% H_2O_2)

Diluted sample in a 100ml volumetric flask, add 50ml of diluent Add 3ml of 30% v/v H2O2 and heated at 70°C for 30 mins on a water bath. Remove the flask from the water bath and allow the flask to cool at room temperature. Diluted to volume with diluent and mixed.

3.8.4. Reduction Degradation (10% Sodium Bisulphate)

Diluted sample in a 100ml volumetric flask, add 50ml of diluent Add 5ml of 10% w/v sodium Bisulphate and heated at 70°C for 1 hours

on a water bath. Remove the flask from the water bath and allow the flask to cool at room temperature. Diluted to volume with diluent and mixed.

3.8.5. Hydrolysis Degradation

Diluted sample in a 100ml volumetric flask, add 20ml of diluent added 50ml of water to disperse and dissolve and heated at 70°C for 1 hour on a water bath. Remove the flask from the water bath, and allow the flask to cool at room temperature and diluted to volume with diluent and mixed.

3.8.6. Thermal Degradation (105°C / 2 hrs)

Sample was exposed at 80°C for 3 hrs and analysed the exposed sample are injected.

3.8.7. Photolytic Degradation

Sample was exposed to sun light for 5Hrs and analysed the exposed samples are injected.

All the degradation study results were recorded and reported (Table 1 & 2).

4. Conclusion

The proposed RP-HPLC, method was suitable methods for the estimation of Metformin and Sitagliptin in drug substances and drug products. All the validation of parameters results of this method met the criteria of ICH guidelines. The developed and validated method was successfully applied for routine analysis of Metformin and Sitagliptin in drug substances and drug products.

Table - 1: Degradation results of Metformin HCl

	Metformin				Peak Purity		
	Sample Weight in mg	Area Counts Injections	Mean Area Count	% Label Claim	%Degradation	Purity Angle	Purity Thresold	Pass/Fail
Control	136.06	5787456	5787456	100.5	-0.2	28.562	0.782	Pass
Acid	135.85	4687413	4687413	75.36	28.52	26.441	0.985	Pass
Alkali	136.24	4589321	4589321	70.58	26.41	28.641	0.952	Pass
Peroxide	136.58	4638415	4638415	74.35	28.36	27.582	0.992	Pass
Reduction	135.92	4689332	4689332	75.58	20.52	28.368	0.982	Pass
Thermal	137.52	4589632	4589632	73.38	21.47	29.328	0.986	Pass
Photo	136.28	4692714	4692714	72.88	20.55	26.258	0.987	Pass
Hydrolysis	135.99	4587131	4587131	77.66	26.24	25.663	0.989	Pass

Table - 1: Degradation results of Metformin HCl

	Sitagliptin				Peak Purity		
	Sample Weight in mg	Area Counts Injections	Mean Area Count	% Label Claim	%Degradation	Purity Angle	Purity Thresold	Pass/Fail
Control	136.06	346954	346954	100.8	-0.5	21.86	1.048	Pass
Acid	135.85	278961	278961	75.5	24.5	26.08	1.046	Pass
Alkali	136.24	287416	287416	76.2	26.2	20.14	1.145	Pass
Peroxide	136.58	276321	276321	77.5	28.5	21.16	1.148	Pass
Reduction	135.92	281245	281245	70.8	27.2	21.25	1.446	Pass
Thermal	137.52	278561	278561	79.2	26.5	22.68	1.146	Pass
Photo	136.28	286412	286412	77.1	22.8	24.18	1.149	Pass
Hydrolysis	135.99	282456	282456	76.3	26.3	22.70	1.414	Pass

5. REFERENCES

1. **Practical HPLC method development**. second edition Lloyd R. Synder, Jpseph J. Kirkland and Joseph L.Glaich pg no: 1-3.
2. Beckett AH, Stenlake JB. **Practical Pharmaceutical Chemistry.** The Anthlone Press London. Vol II 1988; Pg no: 281-307.
3. **Instrumental methods of analysis**. seventh edition by Willard merit dean settle Chromatography pg no: 118-195 & HPLC pg no: 580-654.
4. **Instrumental methods of chemical analysis by B.K.Sharma**. twenty eighth edition 2012 pg no: 286-385.
5. **Vogel's text book of Quantitative chemical analysis.** Sixth edition pg no: 704-714.
6. **Indian Pharmacopoeia.** 2007 vol-1 pg no: 1125-1132.
7. Http/www.wikipedia.com/ Metformin and Sitagliptin
8. Jain N. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Metformin and Sitagliptin in Tablet Dosage Forms. **International Journal of Pharmaceutical Sciences,** 2011; 584-726.
9. Dhaneshwar Sunil R. Validated HPLC Method for Simultaneous Quantitation of Metformin and Sitagliptin in Bulk Drug and Formulation. **International Journal of science**, 1745-1788.