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Original Article

Method development for the Simultaneous Estimation of Metformin and Alogliptin by using RP-HPLC

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ARTICLE INFO	ABSTRACT

A selective and sensitive stability-indicating high-performance liquid chromatographic method was developed and validated for the determination of Metformin and Alogliptin 10 mg of Metformin and Alogliptin was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Metformin and Alogliptin. The isobestic point was taken as detection wavelength. 25 mg of metformin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 1 ml of diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. 10 L of the blank, standard and sample were injected into the chromatographic system and areas for the Metformin and Alogliptin the peaks were used for calculating the % assay by using the formulae. The system suitability parameters for metformin and Alogliptin such as theoretical plates and tailing factor were found to be 2294, 1.27 and 4891 and 1.03, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of metformin and Alogliptin was found in concentration range of 50μg-250μg and 5μg-25μg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.7and 0.8, % RSD for intermediate precision was 0.10 and 0.5 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.

Keywords: Metformin and Alogliptin, RP-HPLC, Acetonitrile (30:70) and Retention time.

1. INTRODUCTION

Qualitative analysis is the identification of elements, species and/or compounds present in sample. Quantitative analysis is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

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Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following. Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by metformin of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose

and fats. Activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors.
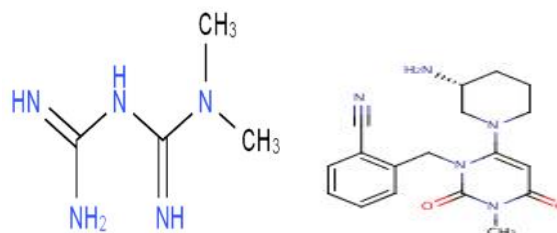

Fig 1: Structure of Metformin & Alogliptin

Metformin administration also increases AMPK activity in skeletal muscle. AMPK is known to cause GLUT4 deployment to the plasma membrane, resulting in insulin-independent glucose uptake. The rare side effect, lactic acidosis, is thought to be caused by decreased liver uptake of serum lactate, one of the substrates of gluconeogenesis. In those with healthy renal function, the slight excess is simply cleared. However, those with severe renal impairment may accumulate clinically significant serum lactic acid levels. Other conditions that may precipitate lactic acidosis include severe hepatic disease and acute/decompensated heart failure.

2. MATERIALS AND METHODS

2.1 Chromatographic conditions

Column : X Bridge C18 column (4.6×150mm), 5µ

Mobile phase ratio : Buffer: Methanol: ACN (20:60:20%v/v/v)

Detection wavelength : 290 nm

Flow rate : 1.0ml/min

Injection volume : 20µl

Column temperature : Ambient

Auto sampler temperature : Ambient

Run time : 15 min

Retention time : 2.365 & 3.907 min
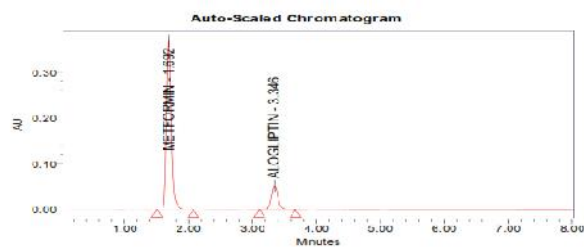
Fig 2: Chromatogram showing trial-5 injection

The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

2.2 Preparation of phosphate buffer

2.95 grams of KH_2PO_4 and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicated and filtered.

2.3 Preparation of mobile phase

Mix a mixture of above buffer 20 ml (30%) and 60 ml of methanol (HPLC grade-60%) and Acetonitrile (20%) degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

2.4 Preparation of the individual Metformin standard preparation

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

2.5 Preparation of the individual Alogliptin standard preparation

10 mg of Alogliptin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 1 ml of diluent and sonicated to

Dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further

pipette out 1 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

2.6 Preparation of the Metformin and Alogliptin standard and sample solution

Sample solution preparation

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

Standard solution preparation

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

2.7 Analytical Method Validation

Specificity

The system suitability for 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.

2.8 Linearity

2.8.1 Preparation of stock solution

25 mg of metformin and 10 mg of Alogliptin working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 1 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

2.8.2 Preparation of Level – I (60ppm of metformin and 1.5 ppm of Alogliptin)

0.6 And 0.15 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

2.8.3 Preparation of Level – II (70ppm of metformin and 1.75ppm of Alogliptin)

0.7 And 0.17 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

2.8.4 Preparation of Level – III (80ppm of metformin and 2.0ppm of Alogliptin)

0.8 And 0.2 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

2.8.5 Preparation of Level – IV (90 ppm of metformin and 2.25ppm of Alogliptin)

0.9 And 0.225 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

2.8.6 Preparation of Level – V (100 ppm of metformin and 2.5ppm of Alogliptin)

1.0 And 0.25 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

2.9 Accuracy

2.9.1 Preparation of standard stock solution

25mg of metformin and 10 mg of Alogliptin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 1ml 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 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

2.9.2 for preparation of 50% solution (with respect to target assay concentration)

15 mg of metformin and 5 mg of Alogliptin working standard were accurately weighed and transferred into a 10 ml clean dry volumetric flask add about 1ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock Solution).Further pipette out 10 ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

2.9.3 For preparation of 100% solution (with respect to target assay concentration)

25 mg of metformin and 10 mg of Alogliptin working standards were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 1 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 1ml of above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

2.9.4 For preparation of 150% solution (with respect to target assay concentration)

30 mg of metformin and 15 mg of Alogliptin working standards into a 10ml clean dry volumetric flask add about 1 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

3. RESULTS AND DISCUSSIONS

3.1 Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of metformin and Alogliptin was obtained and the isobestic point of metformin and Alogliptin showed absorbance's maxima at 290 nm. The spectrums are shown in Fig. 1.
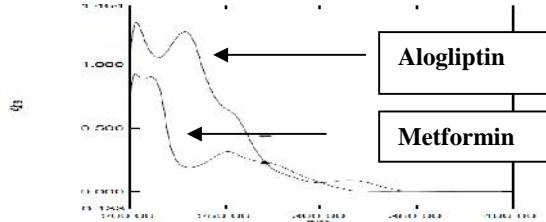
Fig 3: Spectrum showing overlapping spectrum of MET and ALO

**Table 1: Linearity Level of Metformin and Alogliptin**

S.No	Linearity Level	Concentration	Area (Metformin)
1	I	1.5ppm	47154
2	II	1.75 ppm	65627
3	III	2.0ppm	79499
4	IV	2.25 ppm	94612
5	V	2.5 ppm	100213
Correlation Coefficient			0.999

Sl.NO	Leniarity level	Concentration	Area(Alogliptin)
1	I	60 ppm	56472
2	II	70ppm	73841
3	III	80ppm	92655
4	IV	90ppm	111541
5	V	100ppm	130567
Correlation Coefficient			0.999
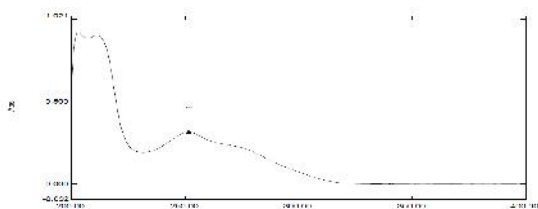
Fig 4: Spectrum showing wavelength of Metformin
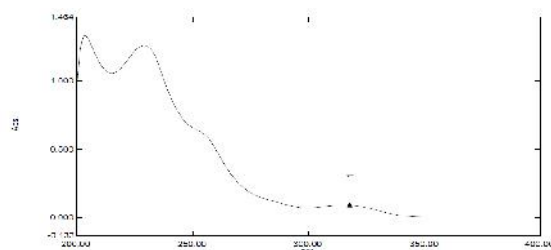
Fig 5: Spectrum showing wavelength of Alogliptin

The chromatographic method development for the simultaneous estimation of metformin and Alogliptin were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of metformin and Alogliptin in API and pharmaceutical dosage form by RP-HPLC method.

Optimized chromatographic conditions for simultaneous estimations of Metformin and Alogliptin by RP-HPLC method

Column   :   X Bridge RP C$_{18}$ 4.6×50 mm 3.7 µm

Column temperature   :   Ambient

Wavelength   :   290 nm

Mobile phase ratio   :   40:20:40 methanol: ACN **:** phosphate buffer pH 7

Flow rate   :   1 ml/min

Auto sampler temperature   :   Ambient

Injection volume   :   10µl

Run time   :   10.0 minutes

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank.
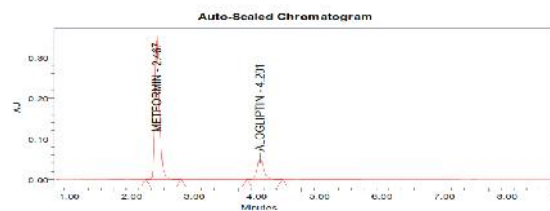
Fig 6: Chromatogram showing sample injection

Linearity : The linearity study was performed for the concentration of 60 ppm to100 ppm of metformin 1.5 ppm to 2.5 ppm of Alogliptin level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient
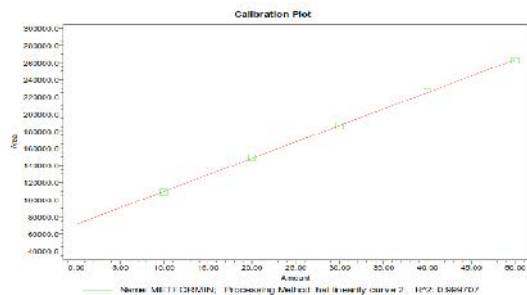
Fig 7: Showing calibration graph for Alogliptin Accuracy

Metformin $r^2 = 0.999$

The accuracy study was performed for 50%, 100% and 150 % for metformin and Alogliptin. Each level was injected in triplicate int o chromatographic system. The area of each level was used for calculation of % recovery.

Table 2: Showing accuracy results for Alogliptin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	484733	0.5	0.99	99.53%	99.47%
100%	967998	1.0	1.05	99.38%	
150%	145437	1.5	1.495	99.52%	

The accuracy study was performed for % recovery of Metformin and Alogliptin. The % recovery was found to be 99.18% and 99.91% respectively (NLT 98% and NMT 102%).

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