An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND SITAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ARTICLE INFO

Key Words

Metformin ,Sitagliptin, system suitability, linearity, precession, assay, LOD, LOQ.

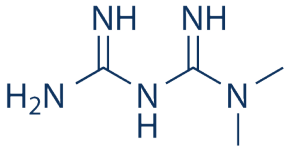
ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of metformin and sitagliptin in bulk and formulation dosage.A column of ODS(250mm 4.6mm; i.d and 5µ particle size) was used. The mobile phase comprises of 0.02M di potassium hydrogen orthophosphate buffer (pH adjusted to 3.3) and acetonitrile in the ratio of 40:60 (v/v). The flow rate was 1.0 ml/min and the effluents were monitored at 260 nm. The retention time for metformin was 3.0 min and sitagliptinwas 3.9min.The detection concentration was linear over 125-750ppm for metformin and 12.5-75ppm for sitagliptin. Regression equationsof metformin and sitagliptin were found to be y = 25883x + 19711 and y = 27696x + 6046 respectively with regression co-efficient 0.999. The developed method was successfully validated in accordance to ICH guidelines. Hence, this method can be conveniently adopted for the routine analysis in quality control laboratories.

INTRODUCTION:

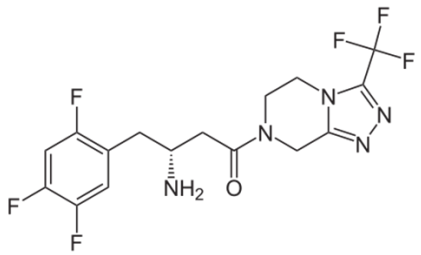
Metformin is an oral antidiabetic drug in thebiguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function. Its use in gestational diabetes has been limited by safety concerns although at least one study has been conducted which showed no concerns for children prenatally exposed to Metformin up to 2 years of age. It is also used in the treatment of polycystic ovary syndrome, and has been investigated for other diseases where insulin resistance may be an important factor. Metformin works by suppressing glucose production from three-carbon molecules (like propionic acid, a byproduct of dietary fibre fermentation in the large intestine and pyruvate, a byproduct of glucose breakdown in the muscles) by the liver[1,2]. Chemically metformin is N,N-dimethylbiguanide with chemical formula $C_4H_{11}N_5$and molecular weight 129.16 g/mol.

Figure 1: Structure of metformin



Sitagliptin (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 marketed byMerck & 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. The benefit of this medicine is its fewer side effects (e.g., less hypoglycemia, less weight gain) in the control of blood glucose values. While safety is its advantage, efficacy is often challenged as it is often recommended to be combined with other agents such as Metformin[1,2]. Sitagliptin is chemically (2R)-4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine with molecular formula $C_{16}H_{15}F_6N_5O$ and molar mass 407.31 g/mol.

Figure 2: Structure of sitagliptin



Few HPLC methods were developed for estimation of metformin and sitagliptin[3-12]. The present developed method was successfully validated in accordance to ICH guidelines [13].The results of the study showed that the proposed RP-HPLC method is useful for the routine determination of metformin and sitagliptin in bulk drug and in its pharmaceutical dosage form.

MATERIALS AND METHODS:

Metformin and sitagliptinwere obtained as a gift sample from Hetero Drugs Ltd. Hyderabad. Acetonitrile, methanol, potassium dihydrogen phosphate and ortho-phosphoric acid used were of analytical grade. Commercially available metformin capsules (Act Metformin®-500 mg) and sitagliptin (Janvia®-100mg) were procured from local market.

INSTRUMENTS:

Quantitative HPLC was performed on Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing powered with Empower-2 Software. An ODC column of 250mm 4.6mm: i.d and 5µ particle size was used.PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for UV measurements.

Preparation of standard solution:

Accurately weighed and transferred 50mg of Metformin and 5mg of Sitagliptin working Standards into 10ml clean dry volumetric flasks, add 3/4 ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml and made up to 10ml and final concentration is 500 µg/ml and 50 µg/ml.

Preparation of working standard:

Ten tablets of formulation were accurately weighed, the average weight of

tablets was found and crushed to a fine powder. From the triturate of ten tablets, an amount equivalent to 2500mg was accurately weighed and transferred in to 100ml volumetric flask and make up to the mark with diluent. The solution was sonicated for 25 min and filtered through whattman filter paper No.41.From the clear solution pipette out 0.2ml and transfer into a 10 ml volumetric flask and made up to the mark with diluent and final concentration is 500 µg/ml and 50 µg/ml.

Preparation of mobile phase:

Prepare a mixture of 40ml buffer and 60ml acetonitrile, degas in ultrasonic water bath for 5min.Filter through 0.45m under vaccumfiltration.

Preparation of Buffer (0.01 KH_2PO_4):

Accurately weigh 1.36gm of Potassium di-hydrogen ortho phosphate in a 1000ml of volumetric flask, add about 900ml of milli-Q water and degas to sonicate. Finally make up the volume with water and pH adjusted to 3.3 with ortho phosphoric acid solution.

EXPERIMENTATION:

System Suitability:

The system suitability studies were evaluated by comparing with standard chromatogram and by obtaining the parameters retention time, column efficiency and tailing factor. All the system suitability parameters are within range and satisfactory as per ICH guidelines [13].

Linearity:

Accurately weighed 50mg of Metformin and 5mg of Sitagliptin working standards transferredinto 10ml clean dry volumetric flasks, added 3/4 ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents to obtain final concentration of 5000 µg/ml and 500 µg/ml.Six linear concentrations of Metformin and Sitagliptin (125ppm to 750ppm) were prepared and injected. Regression equation of Metformin and Sitagliptin are found to be, y = 25883x + 19711 and y = 27696x + 6046.The regression co-efficient was 0.999.

Assay studies:

Six homogeneous samples of both sample and standard were injected. Percentage assay of the drug in the formulation was estimated. The average %assay was calculated and found to be 99.87% and 100.16% for metformin and sitagliptin respectively. The assay data was tabulated in Table 3.

Precision:

Interday precision was performed with 24 hrs time lag and the %RSD obtained for Metformin and sitagliptin were 1.92% and 1.76 %.(Table 5)

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD* and LOQ** of the drug were calculated using the following equations designated by International Conference on Harmonization (ICH) guideline [13] .

* LOD = 3.3 × /S and **LOQ = 10 × κS

Where p= the standard deviation of the response * S = Slope of calibration curve

LOD for Metformin and Sitagliptin were found to be 2.51 and 0.72 respectively. Limit of Quantification was calculated by Metformin and Sitagliptin method and LOQ for Metformin and Sitagliptin were found to be 7.62 and 2.18 respectively.

Table 1: System suitability data

Property	Metformin	Sitagliptin
Retention time (t_R)	3.0± 0.3 min	3.9±0.3min
Theoretical plates(N)	6477 ± 163.48	7979± 163.48
Tailingfactor (T)	0.86 ± 0.117	1.34± 0.117

Figure 3: Optimized Chromatogram of Metformin and Sitagliptin

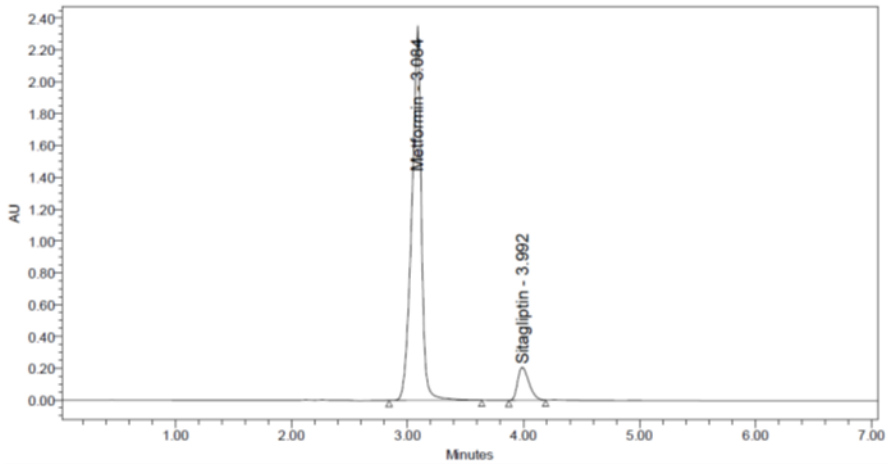


Table 2: Calibration data of metformin and Sitagliptin

S.No	Concentration Metformin(µg/ml)	Response	Concentration Sitagliptin(µg/ml)	Response
1	0	0	0	0
2	125	3481464	12.5	352868
3	250	6645306	25	689638
4	375	10066263	37.5	1052360
5	500	13408035	50	1405358
6	625	16372442	62.5	1753133
7	750	19348128	75	2059078

Figure 3: Calibration Curve of metformin

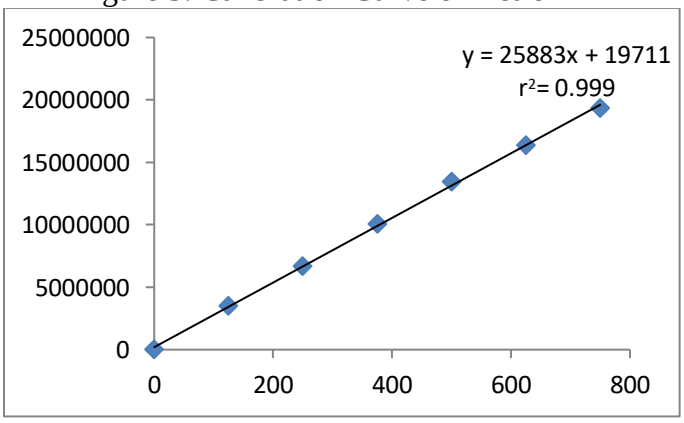


Figure 4: Calibration Curve of sitagliptin

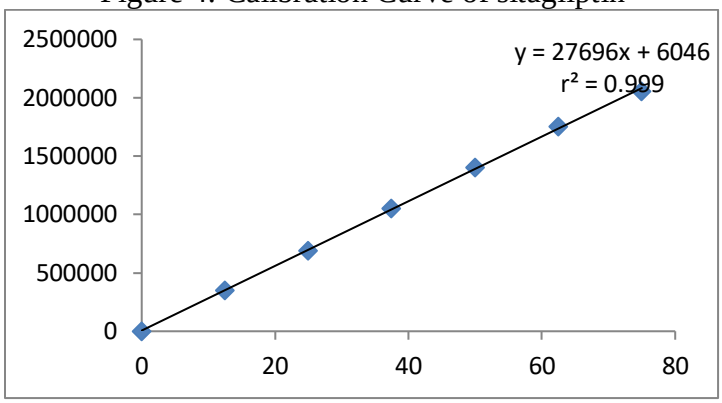


Table 3: Assay data metformin and sitagliptin:

S. No.	Metformin %Assay	Sitagliptin %Assay
1	99.24	100.13
2	100.61	99.55
3	99.61	100.92
4	100.53	99.50
5	100.16	100.54
6	99.07	100.31
AVG	99.87	100.16
STDEV	0.66	0.56
% RSD	0.66	0.56

Figure 6: Standard assay chromatogram

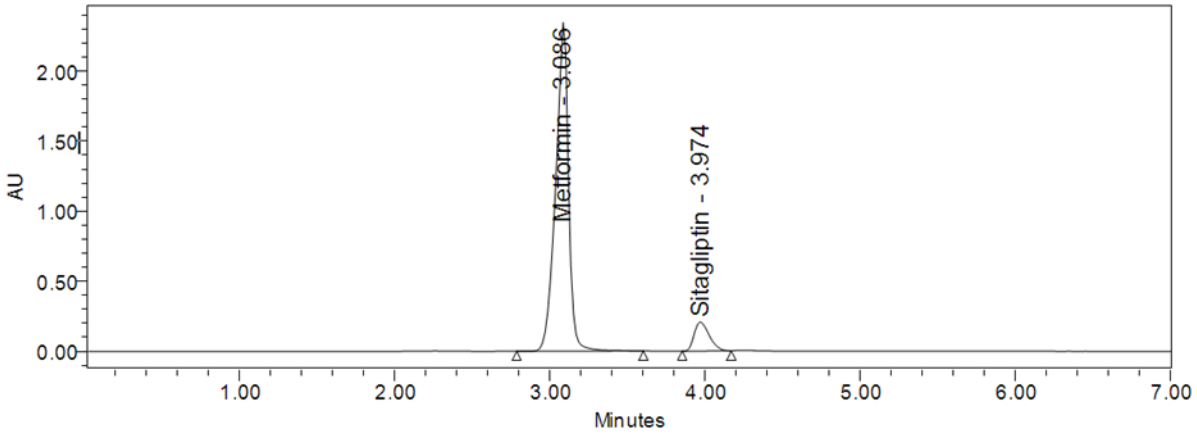


Table 5: Inter day precision studies

S. No.	Metformin	Sitagliptin
1	13541278	1387585
2	13515981	1412087
3	13030796	1353382
4	12991186	1350963
5	13056331	1357619
6	13087151	1362676
Mean	13203787	1370719
Std. Dev.	253708	24178.3
%RSD	1.92	1.76

Table 6: LOD Results for Metformin and Sitagliptin

S.No	Drug Name	Conc. (µg/ml)	RT(min)	Area (μV^* sec)
1	Metformin	2.51	3.047	56211
2	Sitagliptin	0.72	3.970	5016

Figure 7: LOD Chromatogram of metformin and sitagliptin

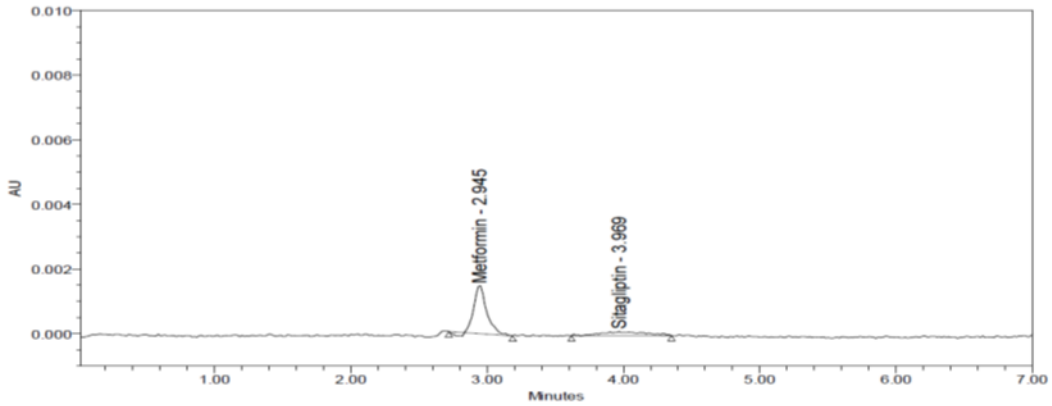
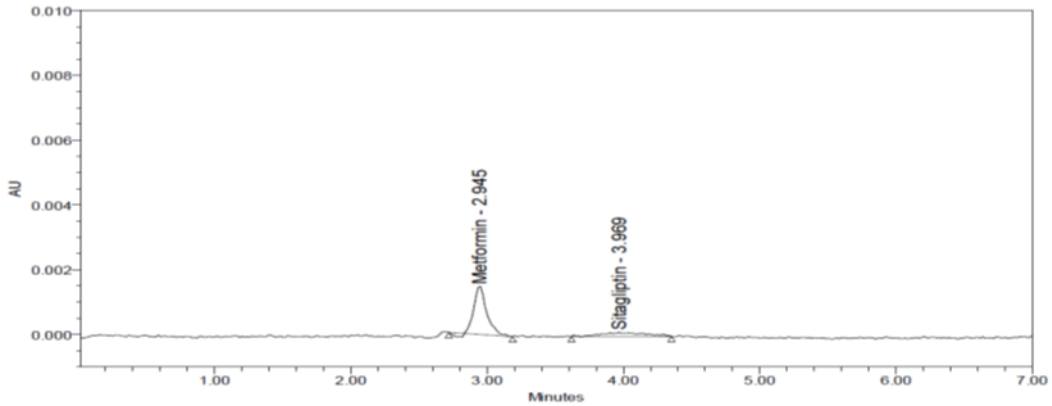


Figure 8: LOD Chromatogram of metformin and sitagliptin



LOQ: Limit of Quantification was calculated by Metformin and Sitagliptin method and LOQ for Metformin and Sitagliptin were found to be 7.62 and 2.18 respectively**.**

Table 7: LOQ Results for Metformin and Sitagliptin

S.No	Drug Name	Conc. (µg/ml)	RT(min)	Area (µV* sec)
1	Metformin	7.62	3.047	56211
2	Sitagliptin	2.18	3.970	5016

Figure 9: Chromatogram of Limit of Quantification

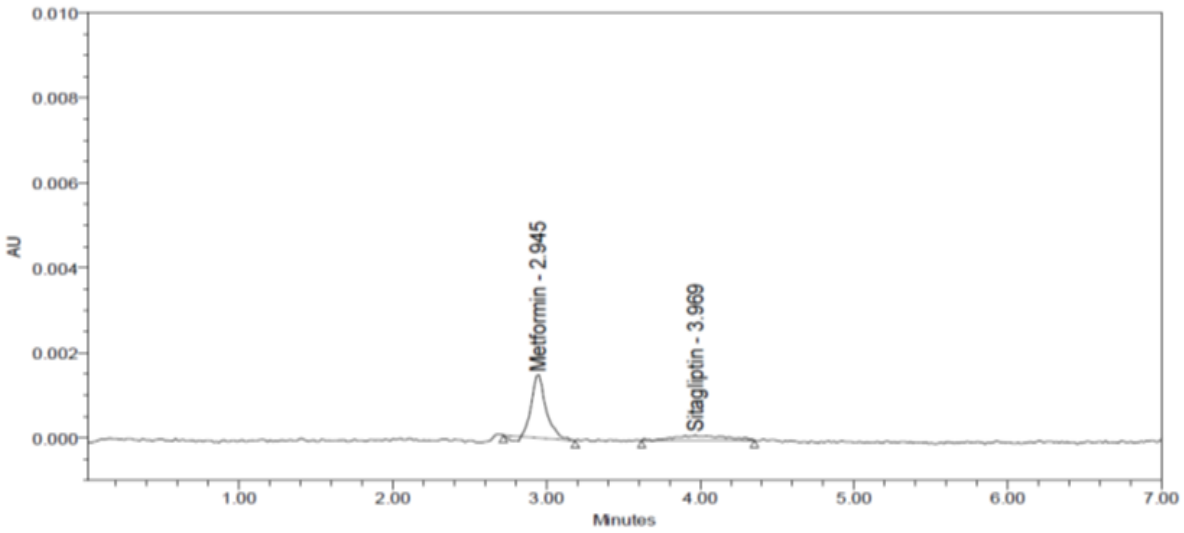


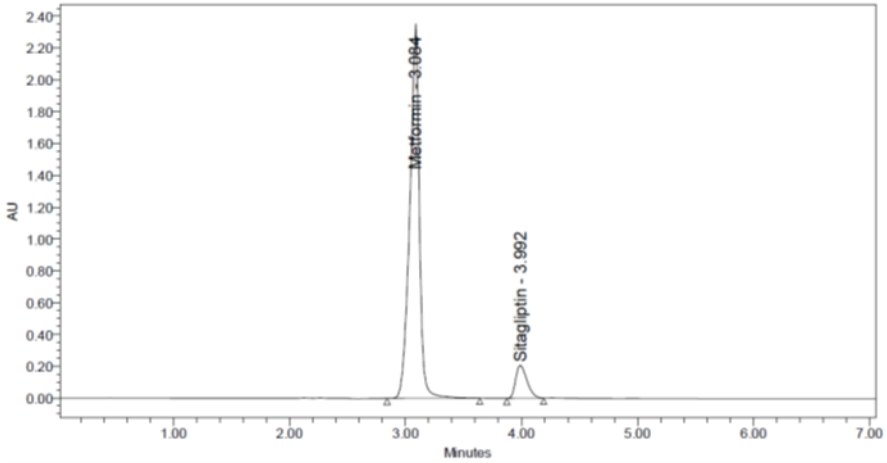
Table 8:Robustness results for Metformin and Sitagliptin

S.No	Robustness condition	Metformin %RSD	Sitagliptin %RSD
1	Flow minus	0.3	0.0
2	Flow Plus	0.5	0.6
3	Mobile phase minus	0.0	0.1
4	Mobile phase Plus	0.3	0.2
5	Temperature minus	0.2	0.1
6	Temperature Plus	0.5	0.1

Table 6: Detection characteristics of metformin and sitagliptin:

Parameters	Metformin	sitagliptin
Calibration range (µg / ml)	125-750ppm	12.5-75ppm
Optimized wavelength	260nm	260nm
Retention time	3.0min	3.9min
Regression equation (Y)	y = 25883x + 19711	y = 27696x + 6046
Correlation coefficient(r^2)	0.999	0.999
Precision (% RSD)	1.92%	1.76%
% Assay	99.87%	100.16%
Limit of Detection (µg / ml)	2.51ppm	0.72ppm
Limit of Quantitation (µg / ml)	7.62ppm	2.18ppm

Figure 10: Typical chromatogram of metformin and sitagliptin:



ROBUSTNESS:

Small deliberatechanges in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines [13].

RESULTS AND DISCUSSION:

From the typical chromatogram of drugs as shown in Figure 10, the retention time for metformin is 3.004min and sitagliptin is 3.992min. The mobile phase comprises of 0.02M di potassium hydrogen orthophosphate buffer (pH adjusted to 3.3) and acetonitrile in the ratio of 40:60 (v/v). The flow rate was 1.0 ml/min and the effluents were monitored at 260nm.Over 1.0 ml/min gradient mode of separation which was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r=0.9999) was observed between the concentration range of 125-750 ppm for metformin and 12.5-75ppm for sitagliptin. Low values of standard deviation are indicative of the high precision of the method. (Table 6). The assay of metformin was 99.87% and sitagliptin was 100.16% (Table 3). The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the formulation. The limit of detection (LOD) and limit of quantification (LOQ) for metformin were found to be 2.51 and 7.62 ppm; for sitagliptin were 0.72 and 2.18ppm respectively.(Table 6, Table 7). This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablets dosage form of the drugs within a short analysis time.

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