Validated RP-HPLC method for simultaneous estimation of metformin hydrochloride and sitagliptin phosphate in bulk drug and pharmaceutical dosage form

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ABSTRACT

A new simple isocratic RP-HPLC method was developed for the simultaneous estimation of Metformin Hydrochloride and Sitagliptin Phosphate in tablet formulation. The separation was achieved on SUPELCO C-18 analytical column (150cm ×4.6mm I.D., 3µm particle size) in isocratic elution mode with the mobile phase consisting of 10mM Potassium dihydrogen ortho phosphate (pH:3.00): Acetonitrile in the ratio of 65:35 v/v and the column was maintained at 25°C. The detection of eluent from the column was detected using UV detector at 256nm and the flow rate was maintained at 1.0 ml/min. The proposed method has permitted the quantification of Metformin Hydrochloride and Sitagliptin Phosphate in the linearity range of 100-300µg/ml and 10-30µg/ml. The method was validated in terms of system suitability, specificity, linearity and precision. The proposed method can be successfully applied in the quality control of bulk and pharmaceutical dosage forms.

Key words: Metformin Hydrochloride, Sitagliptin Phosphate, HPLC, Isocratic elution, Method validation.

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INTRODUCTION

Metformin, N,N-Dimethylimidodicarbonimidic diamide (Fig.1), is an oral antidiabetic drug in the biguanide class. It is the drug of choice for the treatment of type 2 diabetes. 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.[1][2][3][4]
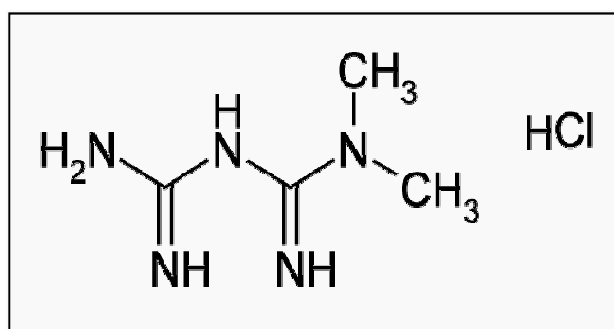

Fig.1. Metformin Hydrochloride

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Sitagliptin,(R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3a]pyrazin7(8H)yl]-1-(2,4,5-trifluoro phenyl) butan-2-amine(Fig. 2), is an oral antihyperglycemic (antidiabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class.[5][6][7][8]
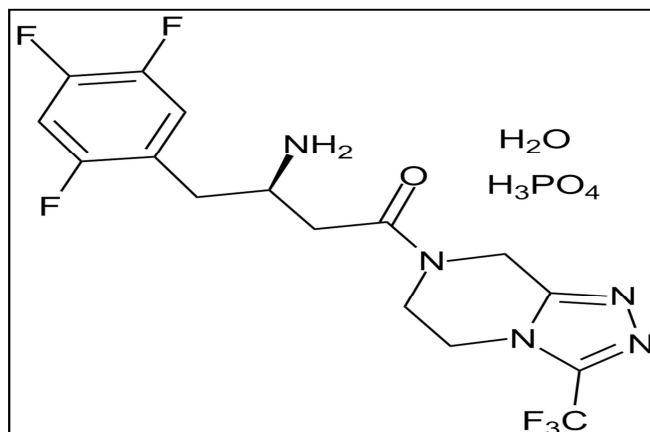

Fig.2. Sitagliptin Phosphate

MATERIALS AND METHODS

Materials and Methods
Chemicals: Pure Standard of Metformin Hydrochloride and Sitagliptin Phosphate were obtained from CIPLA Ltd (Mumbai, India.). Janumet® tablets were purchased from the local medical store.[9] HPLC grade acetonitrile, OPA and Potassium dihydrogen ortho phosphate were obtained from Sigma Aldrich. High purity deionised water was obtained from a Millipore, Milli-Q purification system. All solvents and reagents were of analytical grade.

Instrumentation
Shimadzu HPLC equipped with UV detector was used throughout the analysis. The data was acquired using Lab-Solutions software. The analytical column Supelco C18 (150cm x4.6mm; 3μ) was used as a stationary phase. Sartorious Electronic balance was used for weighing the contents. The instrumental settings were a flow of 1.0mL/min the injection volume was 20μL. Column oven temperature was maintained at 25^0C.

Optimization of Chromatographic Conditions
The chromatographic conditions were optimized by different means (Using different column, different buffer and different mode of HPLC run).

Chromatographic Conditions
The chromatographic elution was carried out in isocratic mode using a mobile phase consisting of 10mM Potassium dihydrogen Ortho-phosphate (pH: 3.0, adjusted with OPA) : Acetonitrile in the ratio of 65:35 v/v and the column was maintained at 25°C. The analysis was performed at a flow rate of 1.0mL/min with a run time of 10 min. The eluent was monitored at wavelength of 265nm. The 20μl volume of sample was injected by autosampler.

Preparation of Mobile phase
The solvent of a mixture of 10mM Potassium dihydrogen ortho-phosphate (pH: 3.0, adjusted with OPA): Acetonitrile used for the preparation of mobile phase in the ratio of 65:35 (v/v). The contents of the mobile phase were filtered before use through a 0.45μm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 ml/min.

Preparation of Standard solutions
A stock solution of Metformin hydrochloride and Sitagliptin phosphate was prepared by dissolving 100mg and 10mg of the drug respectively in 100mL volumetric flask with diluent (Buffer : Acetonitrile i.e. 1:1) to obtain 1000μg/mL. Aliquots of this solution were diluted with diluent to get working standard solutions of Metformin hydrochloride and Sitagliptin phosphate in the concentration ranges 100-300μg/mL and 10-30μg/mL respectively.

Dreparation of Sample solution
Twenty tablets were accurately weighed and crushed in to a fine powder. An amount of powder equivalent to 50mg of Metformin hydrochloride and 5 mg of Sitagliptin phosphate transferred in to 100ml volumetric flask and 20ml 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 0.45µm filter paper. From the above stock solution pipette out 4ml of the solution into a 10ml volumetric flask made up to volume with mobile phase to yield concentration of Metformin hydrochloride (200µg/ml) and Sitagliptin phosphate (20µg/ml). A 20µl sample was injected six times under optimized chromatographic conditions. The peak areas were measured at 256nm.

RESULTS

A typical chromatogram recorded at 256nm is shown in Fig. 3. The retention times of Metformin hydrochloride 3.124mins and Sitagliptin phosphate 7.376mins respectively. The analyte peaks were well resolved.
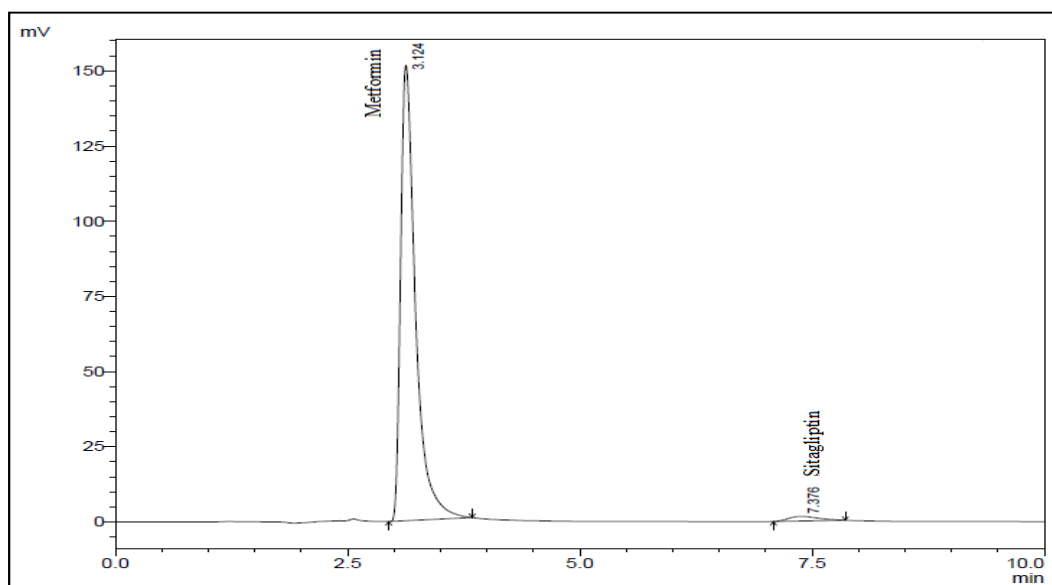

Fig. 3: Typical chromatogram of Metformin and Sitagliptin in Pharmaceutical formulation

Method validation
The developed method was validated as per USP and ICH guide lines. [10]

System suitability
To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factor, theoretical plates and resolution. The values are shown in Table-1.

Parameters	Metformin	Sitagliptin
Retention times (RT)	3.124 min	7.376
HPLC Plate count (USP)	8756	2680
Tailing factor	1.564	1.394
Area	2128878	65134

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.

Linearity
The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 100- 300 µg/ml and 10-30 µg/ml for MET and STG respectively. (Shown in fig 3 and 4) Linear regression data for the calibration curves are given in Table 2.

Table 2: Linear Regression data for the Calibration Curve

Concentration of MET (µg/ml)	Concentration of STG (µg/ml)	Mean peak area of MET	Mean peak area of STG
100	10	967606	20921
150	15	1395479	35144
200	20	1848273	48413
250	25	2252821	62818
300	30	2689389	77722
Slope		8601	2825
Intercept		11035	7506
Correlation co-efficient		0.999	0.999

$y = 8601.8x + 110350$
$R^2 = 0.9998$
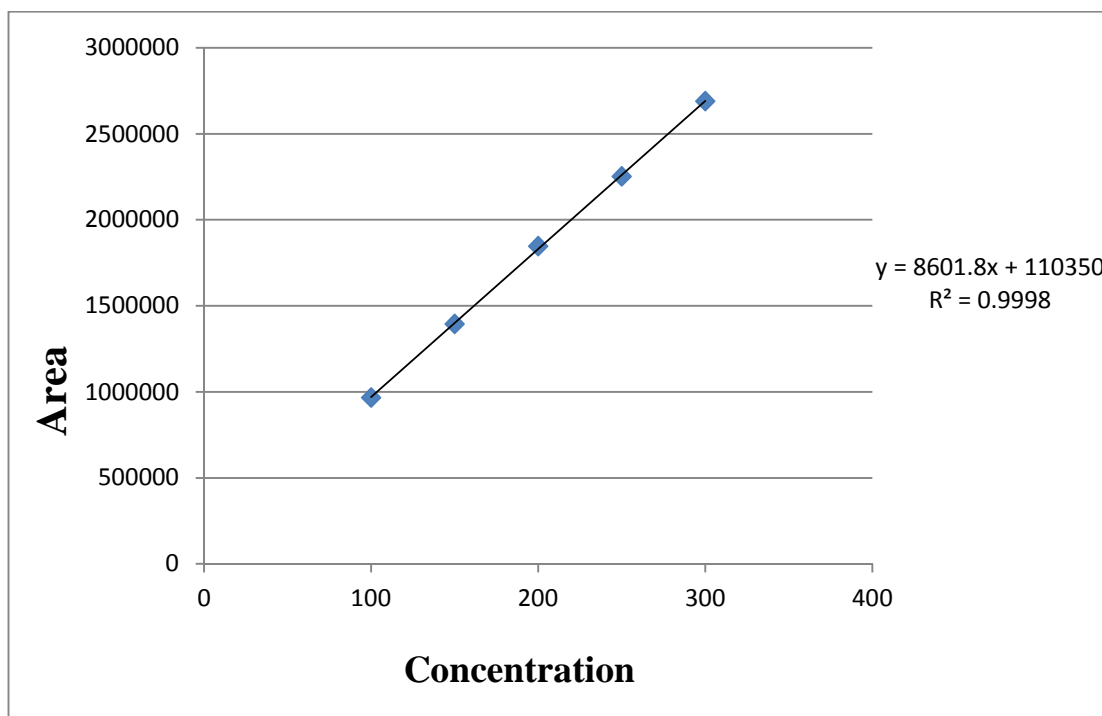
Fig: 3 Linearity curve for Metformin

$y = 2825.5x - 7506.8$
$R^2 = 0.9996$

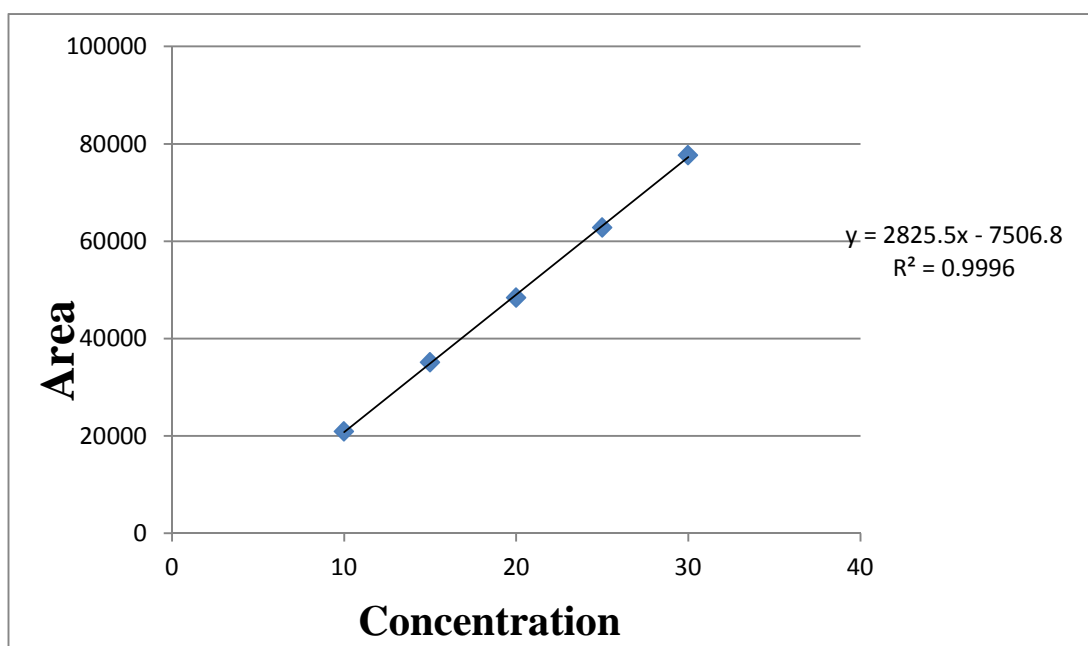**Fig: 4 Linearity curve for Sitagliptin**

Detection limit and Quantification limit

Limit of detection (LOD) and Limit of quantitation (LOQ) of Metformin hydrochloride and Sitagliptin phosphate was conducted. LOD and LOQ were determined by Signal to noise ratio method. The LOD for MET and STG was 24 and 8 µg/ml respectively, while LOQ was 80 and 8 µg/ml respectively.

Precision

Precision was determined as repeatability in accordance with ICH guidelines. The precision was determined by analyzing the samples of MET and STG at concentration of 200µg/mL and 20µg/mL. Determination was performed with six replicates during the same day. The data is shown in table 3.

Table 3: Precision of the proposed HPLC method

Conc. of Metformin (200µg/ml) and Sitagliptin (20µg/ml)	Peak area of Metformin	Peak area of Sitagliptin
Injection 1	2129481	64280
Injection 2	2139003	63113
Injection 3	2125532	63541
Injection 4	2132420	63016
Injection 5	2166502	63135
Injection 6	2151952	64134
Average	**2140815**	**63536.5**
Standard Deviation	**33157.52**	**551.59**
% RSD	**1.54**	**0.86**

CONCLUSION

A developed method for the simultaneous determination of Metformin Hydrochloride and Sitagliptin Phosphate was simple, economic, rapid, precise, accurate and specific. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. The non-interference of tablet excipients makes the method suitable for the simultaneous estimation of these drugs in tablets and hence can be used for routine analysis of Metformin and Sitagliptin in pharmaceutical dosage form.

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