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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Metformin HCl and Sitagiliptin

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ABSTRACT

A reverse phase liquid chromotographic assay method was developed for simultaneous estimation of metformin Hcl and Sitagiliptin from tablet dosage form. The chromatographic separation was achieved on phenomenex C_{18} (250 X 4.6 mm, i.d; 5µ) column with mobile phase consisting Phosphate buffer: Acetonitrile (40:60). The flow rate was 1ml/min and the detection wavelength was 280nm and injection volume was 20µl. The method was validated for linearity, Accuracy, Precision, Specificity, Robustness and Ruggedness.

Key words: RP-HPLC, Method development and validation, Metformin HCL, Sitagliptin.

1. INTRODUCTION

Metformin (Fig -1.) is the first drug of choice for type 2 diabetic mellitus [1-3]. It is an antihyperglicemic drug whic acts by improving the glucose tolearence in patients with type 2 diabeticc mellitus. Sitagliptin (Fig -2.) is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor, which improves glycaemic control by inhibiting DPP-4 inactivation of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). This increases insulin levels, and decreases glucagon levels and post-glucose-load glucose excursion.

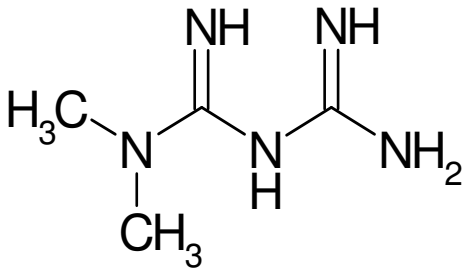

Figure -1: Metfornmin HCL

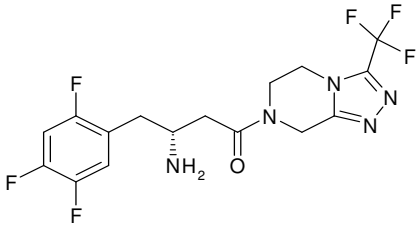

Figure -2: Sitagiliptin

Literature reveiew for metformin and sitagiliptin analysis has been reported as individual ingredients and in combination with other drugs [4-8]. Because no chromatographic method for simultaneous analysis of Metformin Hcl and Sitagiliptin in a combined dosage form, has yet been reported. It was essential to develop a chromatographic method for simultaneous estimation of these two drugs in a tablet formulation. The method described is rapid economical, precise, accurate and can be used for routine analysis of tablets. It was validated as ICH guidelines [9].

2. Experimental

2.1. Drugs and chemicals

Sitagiliptin working standard drugs were received as a gift sample from Tablets India Ltd., Chennai. Metformin HCl and Sitagiliptin tablets were purchased from local market. HPLC Grade methanol and glacial acetic acid are purchased from S.D. Fine chem. Limited.

2.2. Apparatus and chromatographic conditions

LC-10AT HPLC was used for the analysis with UV detection. The separation was carried out using phenomenex C_{18} column (150 X 4.6 mm, i.d; 5µ) with mobile phase containing Phosphate buffer: Acetonitrile (40:60) and elution delivered at a flow rate of 1ml/min. The eluent was monitored at 260nm. The injection volume was 20µl.

2.3. Preparation of standard solution

Weighed accurately about 50 mg of Metformin Hcl and 50 mg of Sitagliptin and transferred in to a clean 100 ml volumetric flask dissolved in few ml of mobile phase and make up to the volume with mobile phase.

Sonicate for 10 minutes and filtered through membrane filter and marked as standard stock solution Pipette out 3ml from the standard stock solution into a clean 50ml standard flask and make up the volume 50 ml with mobile phase and marked as standard stock solution A.

2.4. Preparation of sample solution

Weigh 20 tablets and powdered, weighed accurately a quantity of powder equivalent to 92.4 mg of powder transferred it into a clean 100 ml standard flask. Add few ml of mobile phase and dissolved, make up the volume with mobile phase. The solution is sonicated for 10 minutes and filtered through membrane filter, and marked as sample stock solution.

Pipette out 3ml from the sample stock solution in to a clean 50 ml standard flask and make up the volume 50 ml with mobile phase. So as to give a concentration of 30µg/ml of metformin Hcl and 3µg/ml Sitagliptin.

2.5. Preparation of buffer (0.02M)

1.3609gm of Dipotassium Hydrogen Phosphate in sufficient water to produce 1000ml, pH adjusted to 4.5 with Glacial acetic acid.

2.6. Preparation of mobile phase

Acetonitrile and buffer were mixed in the ratio of 40:60 and filtered through membrane filter and degassed in a sonicator for 10 minutes.

2.7. Selection of wavelength (λ_{max})

The sensitivity of the HPLC method that uses UV detection depends upon the proper selection of the detection wavelength. An ideal wavelength is one that gives good response for the drugs to be detected.

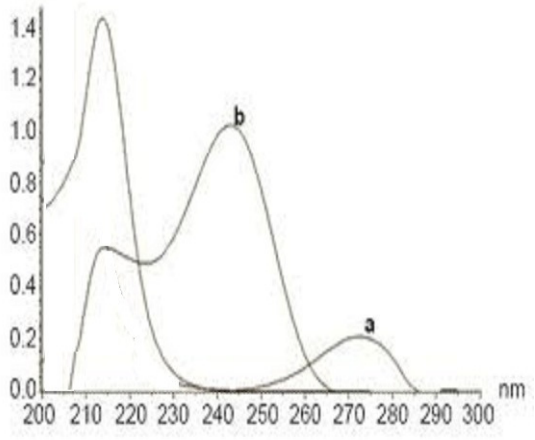

Figure – 3: Overlain spectra of metformin hcl and sitagliptin

Metformin Hcl and Sitagiliptin in diluent (WATER:ACN-50:50), the spectra were scanned on UV-Visible Spectrophotometer separately in the range of 200 to 400 nm against dilluent as blank .The isobestic point of metformin Hcl and Sitagliptin was determined at 260 nm by overlapping the spectrum of metform Hcl and Sitagliptin show in fig -3.

From the UV-Visible Spectrophotometric results, a detection wavelength of 260nm was selected for Metformin Hcl and Sitagiliptin, because at this wavelength they shows good absorbance of both the drugs.

3. RESULTS AND DISCUSSION

The proposed RP-HPLC method 0.02M Potassium Dihydrogen phosphate: Acetonitrile with ratio 40:60, at pH 4.5 was selected as a mobile phase which gives good resolution and good peak shapes shown in fig-1. The flow rate was set at 2.0 ml/min, and the detection was carried out with UV detector at 260nm. At the optimum conditions mentioned above. The total run time required was below 8 mins. The linearity and range was established over the range of 10-50 µg/ml for Metformin and 1-5 µg/ml for Sitagliptin. The correlation coefficient of Metformin and Sitagliptin was found to be 0.9994 and 0.9996.

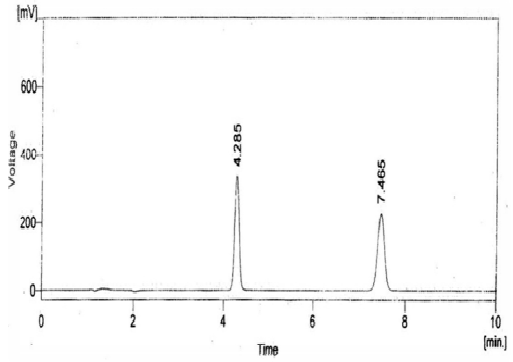

Figure – 4: Standard chromatogram for Metformin Hcl and Sitagiliptin

The accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drug to the blank. The recovery was determined at three levels, viz. 80%, 100%, and 120% of the selected concentrations. The percentage of recovery of Metformin and sitagliptin was found to be 99.59%-100.71%, 99.11%- 101.18% respectively. The low standard deviation values and good recoveries indicate the reproducibility and accuracy of the developed method. As well the % RSD values for precision study also were within acceptable limit.

The limit of detection (LOD) and limit of quantitation (LOQ) for Metformin and Sitagiliptin was 7.12 ng/ml, 1.05 ng/ml and 800.20ng, 99.45 ng/ml, respectively. To determine the robustness

of the developed method experimental conditions were purposely altered and RSD of the peak areas of Metformin and Sitagiliptin were found not greater than 2.0 illustrate the robustness of the method.

3.1. Application of the method to pharmaceutical dosage forms:

The method is sensitive and specific for the quantitative determination of Metformin and Sitagiliptin and also subjected to validation for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. The amount of Metformin Hcl and Sitagiliptin was found to be within the range of 95%-105%.

4. CONCLUSION

The proposed RP-HPLC method for simultaneous estimation of Metformin Hcl and Sitagiliptin in combined dosage form are accurate, precise and reliable. The method, in addition, have the advantages of simplicity, rapidity, economy and less time consuming and may be adopted for routine assay of above mentioned drugs in their tablet formulation.

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