INTERNATIONAL JOURNAL OF PHARMACEUTICAL AND CHEMICAL SCIENCES

Research Article

Devolopment of RP-HPLC Method and It's Validation for Simultaneous Estimation of Sitagliptin and Metformin

Sumithra M[1], Shanmugasudaram MRP, Sankar ASK and Niharika MRS

Department of Pharmaceutical Analysis, School Of Pharmaceutical Sciences, Vels University, Chennai, Tamilnadu, India.

ABSTRACT

A simple, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of Sitagliptin and Metformin. A BDS hypersil C_{18} column (250x4.0mm,5µ) was used with a mobile phase containing a mixture of phosphate buffer (Ph-4) and Acetonitrile and in the ratio of 60:40. The flow rate was 1.0ml/min and effluents were monitored at 260nm and eluted at 2.8min and 2.0min respectively. Calibration curve was plotted with a range from 2-12µg/ml for Sitagliptin and 20-120 µg/ml for Metformin. The assay was validated for parameters like accuracy, precision, robustness and system suitability parameters. The proposed method can be useful in the routine analysis for determination on sitagliptin and metformin.

Key words: Sitagliptin, Metformin, Reverse Phase HPLC.

INTRODUCTION[1]

SITAGLIPTINR)-4-oxo-4-[3-(trifluromethyl)-5,6-dihydro [1,2,4] triaz-olo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine and Metformin is N,Ndimethylimidocarboni-midic diamide are used in the treatment of type 2 diabetes. Structures are shown in fig 1 and 2 respectively. SITAGLIPTIN works to competitively inhibit the emzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP inactivation, they are able to potentiate the secretion of insulin and suppress the release the glucagons by the pancreas. This drives blood glucose levels to normal. Metformin activates AMP-activated protein kinase (AMPK), a liver emzyme 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.

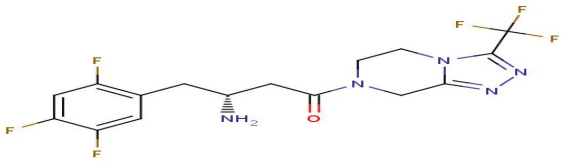

Fig.1: Structure of Sitagliptin

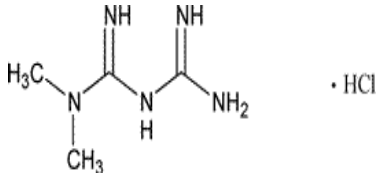

Fig. 2: Structure of Metformin

The literature reveals that there are some of the methods have been reported for sitagliptin UV[2], HPLC[3] and spectrophotometry [4]. For metformin, Development and Validation of RP-HPLC method[5]Simultaneous determination of metformin and

plioglitazone by reverse phase HPLC in pharmaceutical dosage forms[6] . As no HPLC method have been reported for the determination of Sitagliptin and Metformin an attempt was made to report a simple, sensitive, validated and economic method for the determination of Sitagliptin and Metformin

MATERIALS AND METHODS

Reagents:

Acetonitrile and water (HPLC grade). Potassium di hydrogen phosphate and ortho phosphoric acid (GR grade). Commercial samples of tablets containing the drug were purchased from the local pharmacy.

Equipments and apparatus

Different kinds of equipments LIKE Analytical weighing balance, HPLC system (SHIMADZU-SPD 20A), Injector (Rheodyne,20µl), Sonicator, pH meter, Vaccum filter pump, Millipore filtration kit, mobile phase reservoir, Water bath, Sample filtration assembly and glassware's were used throughout the experiment.

Chromatographic conditions

Analysis was carried at 260nm using a BDS hypersil C18 reverse phase column of 250x 4.0mm i.d., 5µm dimensions at ambient temperature. The mobile phase consisted of phosphate buffer(pH 4) : Acetonitrile in the ratio of (60:40 v/v) that was set at a flow rate of1.0ml/min.

Preparation of buffer

Weighed and transferred about 2.87g of potassium di hydrogen phosphate into a beaker containing 1000ml of distilled water and dissolve completely. The pH of the solution was adjusted to 4.0 $\pm$0.01 with orthophosphoric acid and then filtered through 0.45µm membrane filter.

Preparation of Mobile phase

Mobile phase is prepared by mixing 600ml of buffer and 400ml of Acetonitrile. (60:40). The mobile phase is then sonicated using Ultra-Sonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram.

Diluent Preparation

Use the mobile phase as diluent.

Preparation of standard solution

Put approximately 5mg of Sitagliptin and 50 mg of Metformin in 50ml volumetric flask, add mobile phase and sonicate till dissolved. Bring to the volume with mobile phase and mix. Put 1ml of the resulted solution to 10ml volumetric flask. Bring to the volume with mobile phase and mix.

Preparation of sample solution

Transfer precise test portions of powdered tablets equivalent to 5mg of Sitagliptin and 50 mg of Metformin in 50ml volumetric flask, add mobile phase and sonicate till dissolved with intermediate shaking for 10mins. Dilute to volume with mobile phase and mix. Filter a portion of the resulted solution and discard first few ml of the filtrate. Transfer 1.0ml of the above filtered solution into a 10ml volumetric flask, dilute to volume with mobile phase and mix .

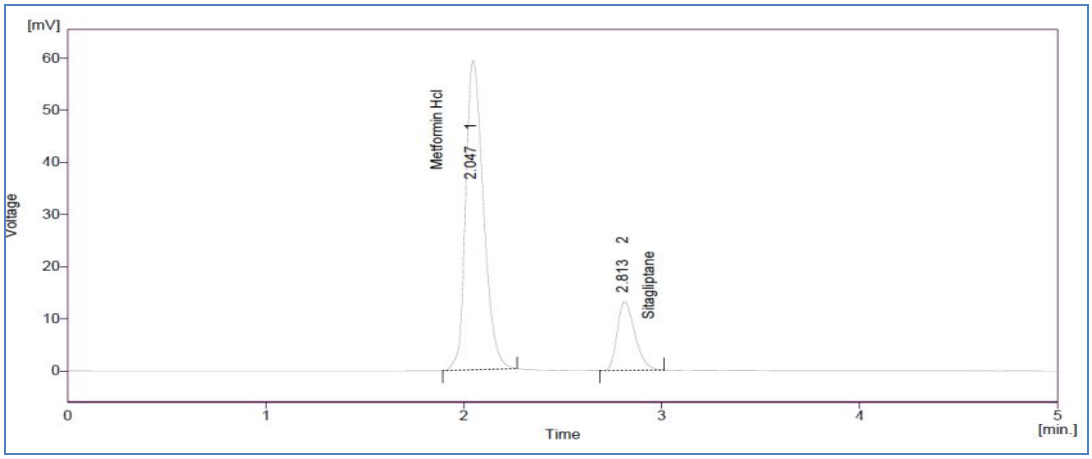

Fig.1: Optimised chromatogram of sitagliptin and metformin by RP-HPLC

METHOD VALIDATION

The proposed method was validated as per ICH guidelines. The dug solutions were prepared as per earlier adopted procedure given in the experiment.

RESULTS AND DISCUSSIONS

A reversed-phase column procedure was proposed as a suitable method for the simultaneous determination of sitagliptin and metformin in combined dosage forms. The chromatographic conditions were optimized by changing the mobile phase composition, p^H, and buffers used in the mobile phase. Different ratios were experimented to optimize the mobile phase. Finally a mixture of Phosphate buffer (p^H 4) in the ratio of 60:40 was used. A typical chromatogram obtained by using the above mentioned mobile phase from 20µl of the assay preparation is illustrated in Fig.3. the retention times of Sitagliptin and Metformin were 2.8 and 2.0 min, respectively. The results discussed in Table 3

The linearity of the method was tested from 2-12µg/ml for sitagliptin and 20-120µg/ml fro metformin. Linearity solutions were injected and the calibration graphs were plotted as peak area of the analyte against the concentration of the drug in µg/ml shown in fig. 4 and 5. in the simultaneous determination of the calibration graphs were found to be linear for both the analytes in the mentioned concentrations and the correlation coefficients for the regression line were 0.9982 and 0,9981 for sitagliptin and metformin respectively.

The accuracy of the method was studied by recovery experiments. The recovery was determined at three levels, viz. 80%, 100%, and 120% of the selected concentrations. Three samples were prepared foe each recovery level. The recovery values for sitagliptin and metformin ranged from 99.16-99.59%, respectively (table 1). The precision of the method was determined from one lot of combined dosage form. The results are shown in (table 2). To determine the robustness of the developed method experimental conditions were purposely alered and RSD of the peak areas of sitagliptin and metformin were found not greater than 2.0 illustrates the robustness of the method.

TABLE I: Accuracy data for Sitagliptin and Metformin

S.No	%concentration of spiked level	Mean %recovery Sitagliptin*	Mean %recovery Metformin*
1	80%	99.28	99.82
2	100%	99.59	99.86
3	120%	99.16	99.53

*Avg of three recoveries.

Table II: precision data for Sitagliptin and Metformin

Injection	Peak area of sitagliptin	Peak area of metformin
1	81.322	370.238
2	80.555	370.983
3	81.442	370.570
4	81.000	370.983
5	82.443	369.257
Mean	81.3542	370.4062
SD	0.699713	0.714349
%RSD	0.86010	0.192856

Table III: Assay of Sitagliptin and Metformin by RP-HPLC

DRUG	Sample peak area	%assay	Avg % assay	Standard deviation	%RSD
Sitagliptin	778.55 770.55 778.05	101.06 99.28 98.96	99.76	1.131	1.133
Metformin	370.983 371883 370.443	99.84 99.94 99.94	99.90	0.057	0.057

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of sitagliptin and metformin. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Sitagliptin and Metformin.

REFERENCES

1. www.rxlist.com
2. Parag Pathade, Md Imran, Viond Bairagi and Yogesh Ahire. Development and validation of stability indicating UV Spectrophotometric method for the estimation of Sitagliptin phosphate in Bulk and target Dosage Form. Journal of Pharmacy Research. 2011;4(3):871-873
3. Tang Yao, L Xiang, Wen Nie, Sun Xu, Yu Min and L Zuo-gang. RP-HPLC determination of sitagliptin phosphate. Chinese Journal of Pharmaceutical Analysis. 2009.
4. Ramzia I El-Bagary, Ehab F Elkady and Bassam M Ayoub. Spectrophotometric Methods for the determination of Sitagliptin and pioglitazone in bulk and dosage forms . International Journal of Biomedical Science.2011;7:55-61
5. Arayne MS, Sultana N, Zuberi MH. Development and validation of RP-HPLC method for the analysis of metformin. Pak J Pharm Sci. 2006;9(3):231-235
6. Sahoo PK, Sharma R and Chaturvedi SC. Simultaneous

Estimation of Metformin Hydrochloride and Pioglitazone Hydrochloride byRP-HPLC Method from Combined Tablet Dosage Form. Indian J Pharm Sci. 2008;70(3):383-386