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Research Article

A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN AND SIMVASTATIN IN TABLET DOSAGE FORM

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ABSTRACT

A simple and rapid reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous determination of sitagliptin (SIT) and simvastatin (SIM) in combined tablet dosage form. The chromatography was carried outby using Hypersil C_{18} (4.6 X 150 mm X 3.5 µm) column (stationary phase) with a mobile phase consisting of a mixture of phosphate buffer: Acetonitrile (30:70v/v), pH adjusted to 3.5 with orthophosphoric acid and the flow rate was kept at 1 ml/min. Analysis was performed at ambient temperature with U.V detection at 254nm. The method was validated for linearity, accuracy (recovery), repeatability, reproducibility,accuracy,LOD and LOQ.The linearity range for sitagliptin and simvastatin was found to be 125-225 µg/ml and 50 – 90 µg/ml respectively. Limit of detection (LOD) and limit of quantitation (LOQ) were 0.5µg/ml and 2.1 µg/ml for sitagliptin and 0.63 and 2.6 µg/ml for simvastatin. The recovery study was carried by standard method and results were in the range of 98.8% to 100% for sitagliptin and 99.0% to 99.42% for simvastatin. The results of the intra and inter day precision studies were found to bewell within the acceptable limits. The developed method was fast, accurate, precise and successfully applied to estimate the amount of sitagliptin and simvastatin in combined tablet dosage form.

Keywords: Sitagliptin, Simvastatin, Reverse phase high performance liquid chromatography

INTRODUCTION

Sitagliptin ((*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4] triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine) an oral antihyperglycemic (for type-II Diabetes) and Simvastatin ((1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate) an hypolipidemic drug (for hypercholesterolemia) were determined using U.V[1-5], HPLC[6-9]. The structures of sitagliptin and simvastatin are as follows:

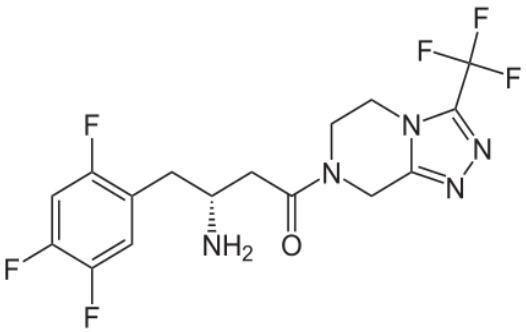

Fig. 1: Chemical structure of sitagliptin

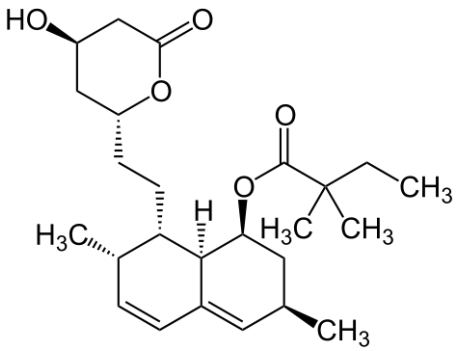

Fig. 2: Chemical structure of Simvastatin

Though not many methods were reported for the sitagliptin and simvastatin estimation in combined form, there is need for better methods for analysis decreasing time, cost, labor and increasing reliability. Few methods in U.V, HPLC were reported on the sitagliptin and simvastatin combination but none of them used Hypersil C18 column, mobile phase of phosphate buffer: acetonitrile in the ratio 30:70 and pH 3.5.

In this paper we report simple, accurate, precise and sensitive R In this paper we report simple, accurate, precise and sensitive Reverse phase high performance liquid chromatography method for simultaneous determination of Sitagliptin and Simvastatin in combined solid oral dosage form. The proposed method is optimized and validated according to ICH guidelines.

MATERIALS AND METHODS

Sitagliptin and simvastatin were supplied kindly from Pharmatrain, Hyderabad. Methanol (HPLC grade), Orthophosphoric acid (AR grade), acetonitrile (HPLC grade), were obtained from Standard chemicals, Hyderabad.

Equipments

RP-HPLC was performed using a Waters 2695 system consisting of a quaternary pump, with an auto-sampler, Empower 2 software, Symmetry C18 (4.6 x 150mm, 3.5µm Make:Hypersil). Eutech pH meter was used. All Calibrated glassware were used for the study.

Preparation of Standard Stock solution

25mg & 10mg of Sitagliptin and Simvastatin working standard were accurately weighed and transferred into a 25 & 10ml clean dry volumetric flask and about 18 & 7ml of mobile phase was added and sonicated to dissolve it completely and the volume was made up to the mark with mobile phase (Stock solution).

Further 1.75ml of Sitagliptin & 0.7ml of Simvastatin were pipetted out from the above stock solution into a 10ml volumetric flask and made up to the mark with mobile phase (175µg/ml-SIT & 70 µg/ml-SIM).

Preparation of mobile phase:

A mixture was prepared of above buffer 300 ml (30%) and 700 ml of Acetonitrile HPLC (70%) and degased in ultrasonic water bath for 5 minutes. It was filtered through 0.45µ filter under vacuum filtration.

Procedure for Sample Preparation

Sample details: Juvisync-100

Label claim: Each tablet contains 128.5mg Sitagliptin phosphate (equivalent to100mg of Sitagliptin and 40mg of Simvastatin).

Mfg. by: Merck & co. inc

20 tablet each containing to100mg of Sitagliptin and 40mg of Simvastatin were weighed and finely powdered. A quantity of powder equivalent to100mg of Sitagliptin was weighed and dissolved in mobile phase. The solution was sonicated and filtered through 0.45µ sieve. From filtrate approximate dilution was done in mobile phase to get a solution of 175 µg/ml of sitagliptin and 70 µg/ml of simvastatin. Such five injections were prepared and injected into the system.

Table 1: Estimation of SIT and SIM in formulation:

Formulation	Labeled Amount(mg)		Amount found(mg)		Percentage assay (%)		%RSD	
	SIT	SIM	SIT	SIM	SIT	SIM	SIT	SIM
Juvisync	100	40	99.8	39.72	99.8	99.3	0.99	0.99

Both the standard and sample preparation was injected separately, and the peak area responses were recorded. The percentage label claim was calculated and given in table-1.

RESULTS AND DISCUSSION

The simultaneous estimation of Sitagliptin and Simvastatin in tablet for was carried out by RP-HPLC using phosphate bufferand acetonitrile as mobile phase in the ratio of 30:70% v/v and Hypersil C18 column as the stationary phase. The results of system suitability parameters such as tailing factor, asymmetry and number of theoretical plates are indicated satisfactory results. The retention time for Sitagliptin and Simvastatin were found to be around 2.5 and 5.1 minutes. The resolution value of more than 2 indicates satisfactory results in quantitative work and the high resolution value obtained indicate the complete separation of the drugs. The linearity was studied in the concentration range from 125-225µg/mL for Sitagliptin and 50-90 µg/mL Simvastatin. The regression co-efficient (R^2) value for Sitagliptin and Simvastatin were found to be 0.9999 and0.9998, respectively. The mean recovery for Sitagliptin was 98.8 to 100.0% and 99.0 to 99.42% for Simvastatin, whichis largely within the 90-110% range that is considered acceptableand it reveals that the method is accurate.

The validation of the proposed method was verified by system precision and method precision. The system precision was evaluated by measuring the peak area responses of Sitagliptin and Simvastatin for five replicate injections of the standard solutions. The method precision was determined by quantifying the sample solutions as per the proposed method. The %RSD was found to be less than 2 for both drug indicates the proposed method is precise. The method was also confirmed by ruggedness study, analyzing the product day to day, analyst to analyst and instrument to instrument. The data for ruggedness of Sitagliptin and Simvastatin are found to be within the acceptance limit. Different validation parameters for the proposed HPLC method for determining Sitagliptin and Simvastatin were summarized in table-2 and chromatogram of Sitagliptin and Simvastatin after separation is shown in fig-3 and fig-4. The result obtained was in agreement with the labeled value of Sitagliptin and Simvastatin in dosage form. The determined validation parameters are in the acceptable ranges.

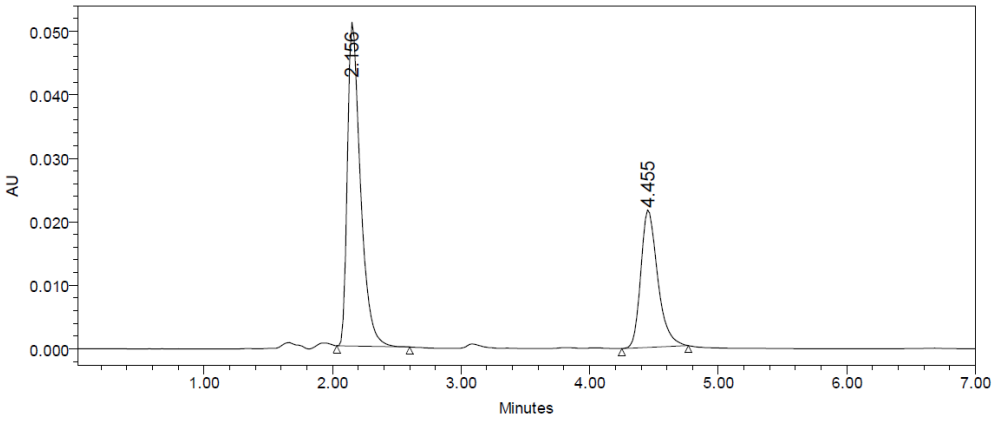

Fig. 3: Chromatogram of Standard solution of SIT 175 µg/mland SIM 70 µg/ml

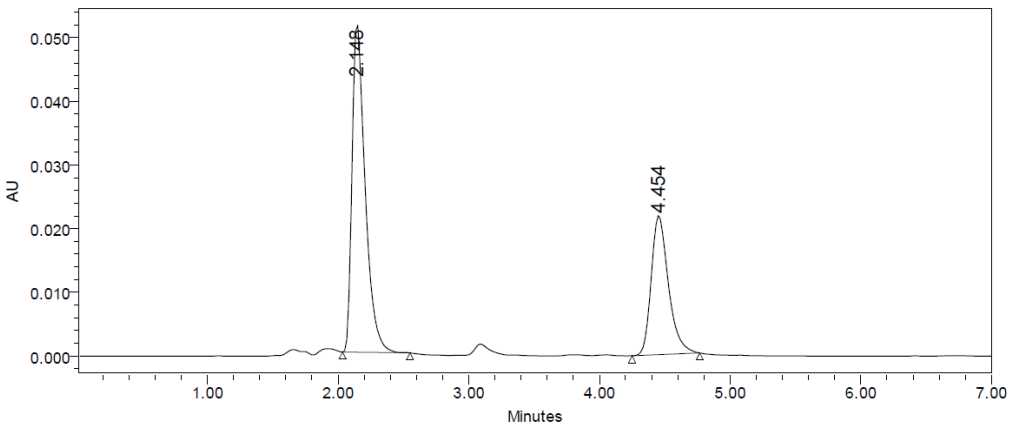

Fig. 4: Chromatogram of SIT and SIMsample

Table 2: Summary of system suitability parameters

Validation parameters	Sitagliptin	Simvastatin
Linearity range (µg/ml)	125-225	50-90
Correlation co-efficient (r^2)	0.99	0.99
LOD (µg/ml)	0.5	0.63
LOQ (µg/ml)	2.1	2.6
Reproducibility (%RSD)	1.98	0.97
Repeatability (%RSD)	0.12	0.19
Peak purity index	1.00	1.00
Accuracy (%)	98.8 – 100.0	99.0 – 99.42
Number of theoretical plates	2112.7	5603
Tailing factor	1.6	1.3

CONCLUSION

The proposed method is simple, accurate, cost effective, less time consuming and the statistical analysis proved that the method is reproducible and efficient for the simultaneous estimation of Sitagliptin and Simvastatin as bulk drugs and in combined pharmaceutical dosage forms without any interference from the excipients.

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