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SIMULTANEOUS ESTIMATION OF THE SITAGLIPTINE AND SIMVASTATIN IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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Abstract

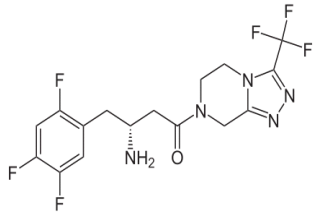
The aim of the present analytical research is to develop a simple, precise, accurate, rapid and economic RP-HPLC method for the simultaneous estimation of the sitagliptine and simvastatin in tablet dosage form. Till to date no accurate and precise RP-HPLC method is developed for the simultaneous estimation of the sitagliptine and simvastatin in tablet dosage form. Waters HPLC 2965 system with an auto injector with PDA detector is used for method development. After the method development method is also be validated for the parameters like system suitability, linearity, accuracy, precession, robustness, limit of detection and limit of quantitation according to ICH guidelines.

KEY WORDS: *Sitagliptine, simvastatin, UV detection, RP-HPLC, etc.*

INTRODUCTION

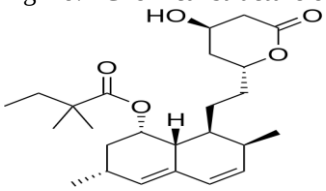
Sitagliptin is Previously identified as MK-0431 and marketed as the phosphate salt under the trade name Sitagliptine) is an oral antihyperglycemic (antidiabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. The chemical structure of the sitagliptin is [1, 2]

Fig No:1 Chemical structure of sitagliptin



Simvastatin is a hypolipidemic drug used with exercise, diet, and weight-loss to control elevated cholesterol, or hypercholesterolemia. Simvastatin is a synthetic derivative of a fermentation product of Aspergillus terreus. The chemical structure of the simvastatin is [3, 4]

Fig No:2 Chemical structure of simvastatin



MATERIALS AND METHOD

Chemicals:

Distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tri ethyl amine, ortho-phosphoric acid etc.

Drugs:

Simvastatin and Sitagliptine, Combination of Simvastatin and Sitagliptine tablets,

Instrumentation:

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Simvastatin and Sitagliptine solutions.

Preparation of buffer:

0.01KH_2PO_4: Accurately weighed and transferred 1ml of Conc.Orthophosphoric acid in a 1000ml of

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Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 0.5ml of Triethylamine then PH adjusted to 3.0 with dil. ortho phosphoric acid solution.

Standard Preparation:

Accurately Weighed and transferred 10mg of sitagliptin and 4mg of simavastatin working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluents , sonicated for 5 minutes and make up to the final volume with diluents.

Sample Preparation:

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 70mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluent.

Optimized chromatographic conditions:

Column Used : BDS (150mm 4.6mm, 5µ)

Buffer used : 0.1%OPA

Mobile phase : Buffer: Acetonitrile (35:65)

Flow rate : 1ml/min

Diluent : Water:Acetonitrile(50:50)

Wavelength : 211
Temperature : 30°C
Injection Volume : 10µl

Assay:

Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference.

Method Validation

System suitability:

10 µL of the standard solution was injected into the chromatographic system and chromatogram was recorded.

Linearity:

Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions of simvastatin and sitagliptine are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 10ppm, 20ppm, 30ppm, 40ppm, 50ppm, 60ppm of simvastatin and 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm of sitagliptine.

Accuracy:

50%: 5 tablets were weighed and calculate the average weight of each tablet then powder weight of 1250mg was transferred into a 100mL volumetric flask, 7mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

100%: 5 tablets were weighed and calculate the average weight of each tablet then powder weight of 2500mg was transferred into a 100mL volumetric flask, 7mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

150%: 5 tablets were weighed and calculate the average weight of each tablet then powder weight of 3750mg was transferred into a 100mL volumetric flask, 7mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Precision:

Sample Preparation:

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 70mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluent.

Robustness:

Small deliberate changes 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.

LOD:

Limit of detection was calculated by intercept method.

LOQ:

Limit of Quantification was calculated by intercept method.

RESULTS

Fig No:3 Optimized Chromatogram

Table No: 1 Data of Assay of Tablet

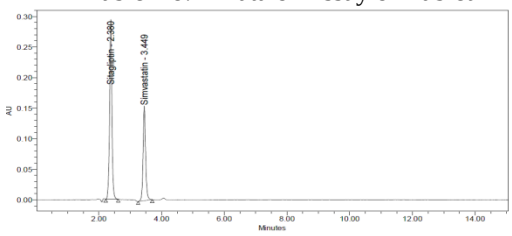
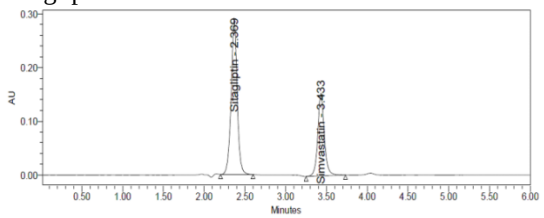


Fig No:4 Assay of Tablet chromatogram of Sitagliptine and Simvastatin



System Suitability:

Table No: 2 Data of System Suitability

Property	Sitagliptine	Simvastatin
Retention time (t_R)	2.3±0.3min	3.4 ± 0.3 min
Theoretical plates (N)	5230± 163.48	9165 ± 163.48
Tailing factor (T)	0.99± 0.117	1.06 ± 0.117
Resolution	7.58	

Fig No: 5 Chromatogram of blank

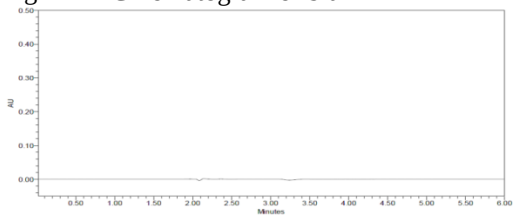
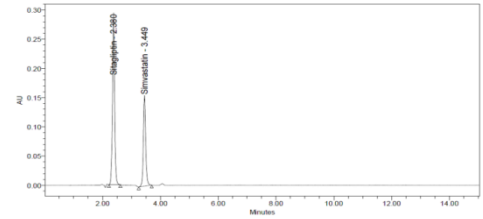


Fig No: 6 Typical chromatogram of sitagliptine and simvastatin



Linearity:

Table No: 3 Calibration data of Sitagliptine and Simvastatin

S. No	Concentration Sitagliptine	Response	Concentration Simvastatin (µg/ml)	Response
1	0	0	0	0
2	25	384185	10	220290
3	50	770146	20	445414
4	75	1170146	30	662012
5	100	1532394	40	862023
6	125	1878928	50	1083943
7	150	2335221	60	1308880

S. No.	Sitagliptine %Assay	Simvastatin %Assay
1	101.57	98.77
2	99.76	99.36
3	99.38	100.59
4	99.49	100.91
5	100.34	99.37
6	101.66	100.73
AVG	100.37	99.96
STDEV	1.0241	0.898
%RSD	1.0	0.9

Fig No:7 Calibration curve of sitagliptine

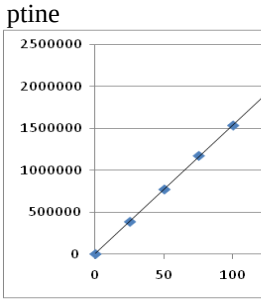
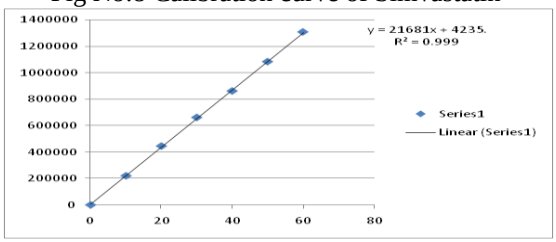


Fig No:8 Calibration curve of Simvastatin



Accuracy:

Table No:4 Accuracy data of Sitagliptine and Simvastatin

Sample	Amount Taken (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Sitagliptine	50	49.91	99.82	0.7
	100	101.83	101.83	0.8
	150	149.22	99.48	1.1
Simvastatin	20	20.07	100.37	0.9
	40	39.728	99.32	0.8
	60	59.76	99.6	0.6

Fig No:9 Accuracy 50% Chromatogram of Sitagliptine and Simvastatin

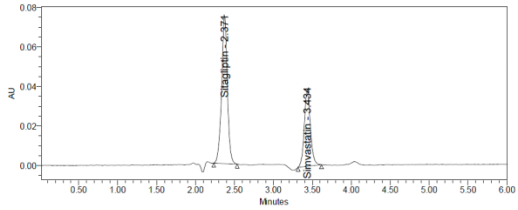


Fig No:10 Accuracy 100% Chromatogram of Sitagliptine and Simvastatin

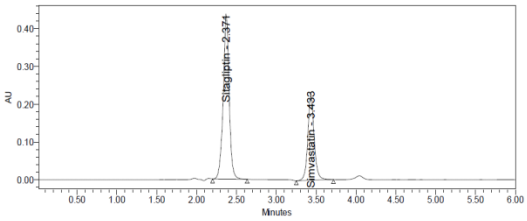
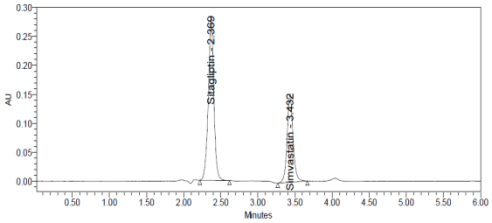


Fig No:11 Accuracy 150%Chromatogram of sitagliptine and simvastatin



Precision:

Intraday precision (Repeatability):
Fig No:12 Repeatability Chromatogram of Sitagiptine and Simvastatin

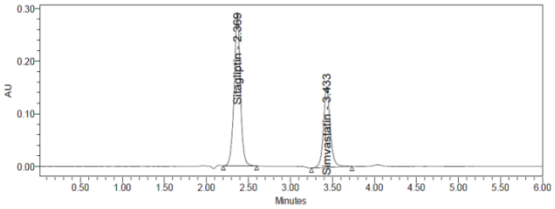
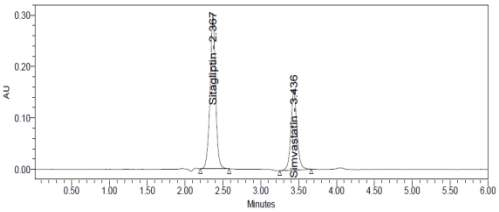


Fig No:13 Inter day precision chromatogram of sitagliptine and simvastatin



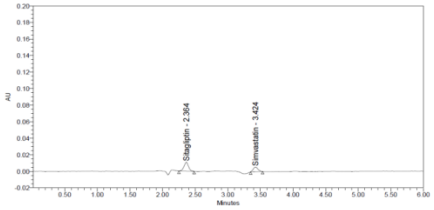
Robustness:

Table No: 5 Robustness data of Sitagliptine and simvastatin

S. NO	Robustness condition	Simvastatin %RSD	Sitagliptine %RSD
1	Flow minus	1.8	1.1
2	Flow Plus	1.4	0.3
3	Mobile phase minus	0.5	2.4
4	Mobile phase Plus	0.3	0.4
5	Temperature minus	0.1	1.8
6	Temperature Plus	0.3	1.6

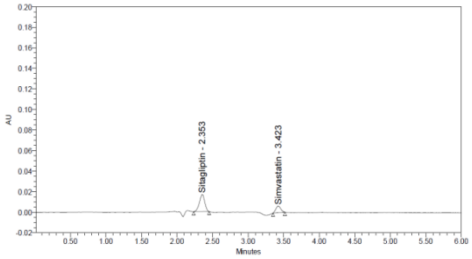
LOD:

Fig No:14 LOD chromatogram of Sitagiptne and Simvastatin



LOQ:

Fig No:15 LOQ chromatogram of Sitagliptine and Simvastatin



Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Sitagliptine and simvastatin in Tablet dosage form. Retention times of Sitagliptine and Simvastatin were found to be 2.3min and 3.4min. %RSD of the Sitagiptine and Simvastatin were and found to be 1.0 and 1.3 respectively. %Recover was Obtained as100.38% and 99.76% for Sitagliptine and Simvastatin respectively. LOD, LOQ values are obtained from regression equations of Sitagliptine and Simvastatin were 0.09, 0.28 and 0.64, 1.95 respectively. Regression equation of Sitagliptine is y = 15368x + 424.6, and of Simvastatin is y = 21681x + 4235. Regression co-efficient was 0.999. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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