Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

https://doi.org/10.36673/AJPAMC.2021.v09.i03.A17

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE DETERMINATION OF SITAGLIPTIN AND SIMVASTATIN IN BULK AND MARKETED DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, rapid, specific and accurate Reverse Phase High Performance Liquid Chromatographic Method have been developed for the validation of Sitagliptin and Simvastatin in bulk as well as in the marketed pharmaceutical dosage form. This separation was performed on a Phenomenex Luna C18 (4.6×250mm, 5µm) particle size column with Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v) as mobile phase at a flow rate of 1.0 ml/ min with UV detection at 245nm; the constant column temperature was Ambient. The run time under these chromatographic conditions was within 10 min. The retention time of Sitagliptin and Simvastatin was found to be 2.12 and 3.53. The calibration plot was linear over the concentration range of 6-14µg/ ml for Sitagliptin and 18-42µg/ ml for Simvastatin with limit of detection 0.63 and 0.80µg/ ml for Sitagliptin and Simvastatin and quantification values 1.8 and 2.40µg/ ml respectively. The mean % assay of marketed formulation was found to be 98.25% and 101.20%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2. The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Sitagliptin and Simvastatin in bulk and marketed pharmaceutical dosage form dosage form.

KEYWORDS

Simvastatin, Sitagliptin, RP-HPLC, Phosphate buffer (pH 4-6), Acetonitrile, ICH validation guidelines, RP-HPLC, Sonicator and Waters HPLC.

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INTRODUCTION

The study of separation, identification, determination and detection of compoundsin the sample is known as Analytical Chemistry. Mainly it involves quantitative analysis and qualitative analysis.

Quantitative analysis is the measurement of amount

of the sample to be analyzed.
Qualitative analysis is the study of purity of the sample.
The instrumental analysis involves the spectroscopic and chromatographic techniques.
Spectroscopy.
Chromatography

Spectroscopic Techniques

Ultraviolet Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, Infrared Spectroscopy, Mass Spectroscopy

Chromatographic Techniques

Gas chromatography, Thin layer chromatography, High performance thin layer chromatography, Paper chromatography, High performance liquid chromatography.

High Performance Liquid Chromatography

It is a type of liquid chromatography that deals with a liquid mobile phase and solid stationary phase, to obtain good separation and flow of mobile phase. HPLC is more advanced than column chromatography. It has high speed, efficiency and high resolution for separation of mixture of compounds, when compared to other techniques.

MATERIAL AND METHODS

The Instrument used was Waters HPLC, with Empower 2 software equipped with UV detector, and solvents used were HPLC grade and the pure drugs like Sitagliptin and Simvastatin were procured from Sura labs, Hyderabad.

Drug profile

Sitagliptin

Sitagliptin competitively inhibits the enzyme dipeptidyl peptidase 4 (DPP-4). Which breaks down the incretins GLP-1 and GIP (gastrointestinal hormones released in response to a meal).By inhibiting the breakdown of GLP-1 and GIP it increases the secretion of insulin and suppress the release of glucagon.

Simvastatin

Simvastatin competitively inhibits hepatic hydroxymethyl-glutaryl coenzyme HMG- CoA reductase, enzyme which catalyzes the conversion of HMG-CoA to mavelonate initiating cholesterol synthesis.

Methodology

Experimental work

The objective of the work was to develop a new method and optimize it by changing the HPLC conditions suitably. The method was developed by trial and error method, where chromatographic conditions were altered.
The trial 5 was found to be accurate as the retention times and the resolution factor for the peaks obtained were good, followed by tailing factor below 2 and theoretical plates above 2000.

Preparation of Mobile Phase

Method Development Trials as Follows

Trial - 1

Observation-Peak of only one compound was observed, the absence of second peak was may be because of its less solubility. So we have gone for further trials.

Trial - 2

Observation**-**The separation of two compounds was obtained but it is improper and the resolution was also not good. So we have gone for further trails.

Trial – 3

Observation-The separation of two compounds was obtained, but the baseline noise was very high and the peaks obtained were asymmetrical. So we have gone for further trails.

Trial - 4

Observation-The separation of two compounds was obtained, baseline was also proper but small asymmetry was observed in the peak. So we have gone for further trails.

Trial - 5

The optimized chromatographic condition as follows:

Mobile phase

Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v)

Flow rate

1.0ml/min

Wave length

245nm

Column

Phenomenex Luna C18 (4.6×250mm, 5µm) particle size.

VALIDATION

The new method which was developed and optimized was now validated according to ICH guidelines.

The method validation studies like Accuracy, Precision, Linearity, Assay, Range, LOD, LOQ, Robustness, Ruggedness and System suitability tests are carried out for the developed new method and the results are tabulated below.

Accuracy

Three different levels such as 50%, 100% and 150% concentration solutions were prepared and the samples are spiked with a known amount of analyte and injected 3 samples for each concentration. Then recovery, % mean recovery and amount found and amount spiked are calculated.

Acceptance criteria: The% mean recovery should be 98-102%

Assay

The sample (marketed dosage form) of the Sitagliptin and Simvastatin was prepared by taking 10mg equivalent weight of 20 tablets and dissolved in mobile phase and injected into HPLC for3 times and the peak areas are recorded. The standard pure drug solutions are also prepared and injected into HPLC for 3 times and the peak areas are recorded.

The Acceptance criteria 98-102%

Precision

The Repeatability and Intermediate precision studies were conducted and the peak area, RSD and %RSD were calculated.

The Acceptance criteria should be <2.

Linearity

The linearity test was conducted by preparing the solutions in the range of 6-14µg/ ml for Sitagliptin and 18-42µg/ ml of Simvastatin and the calibration plot was plotted with peak area Vs Concentration on Y-axis and X- axis and the Co-relation co-efficient was calculated.

The Acceptance criteria should be MT 0.999

LOD and LOQ

The LOD and LOQ were calculated by substituting the values of %RSD and slope from linearity in the respective formula.

System suitability

The standard solutions were prepared and injected into HPLC system 6 times and the plate count and tailing factor are recorded.

The Acceptance criteria should be greater than 2000 for theoretical plate count and less than 2 for tailing factor

Robustness

This is done by changing the flow rate to ± 0.1ml/min and change in the organic composition of the mobile phase to the less and more to the optimized method and observes the changes in the retention time, tailing factor and theoretical plate count.

RESULTS AND DISCUSSION

Discussion

From the literature review, only few methods were reported in RP-HPLC method for the combination of Sitagliptin and Simvastatin. Hence, there is a need for the development of a new method and need of validation.

The present method was validated as per ICH guidelines and the results are tabulated below:

Materials and Instruments used

Table No.1: Instruments, glassware and Chemicals

S.No	Instruments and Glassware's	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detectors
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Lab man

Table No.2: Instruments, glassware and Chemicals

S.No	Chemical	Company
1	Sitagliptin	Sura labs
2	Simvastatin	Sura labs
3	Water and Methanol for HPLC	Lichrosolv (Merck)
4	Acetonitrile for HPLC	Merck

Table No.3: Result for the Assay of Standard

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Sitagliptin	2.120	756985	68958		0.98	7253	1
2	Simvastatin	3.536	2569856	198564	2.06	1.23	8836	1
3	Sitagliptin	2.120	758745	69857		1.05	6530	2
4	Simvastatin	3.537	2598654	195682	2.04	0.99	7270	2
5	Sitagliptin	2.102	756848	69588		1.7	7586	3
6	Simvastatin	3.537	2587454	192541	2.04	1.6	8371	3

Table No.4: Result or the Assay of Sample

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Sitagliptin	2.102	759868	71255		1.7	5689	1
2	Simvastatin	3.537	2458754	215654	2.04	1.6	5362	1
3	Sitagliptin	2.105	759458	72541		1.7	5748	2
4	Simvastatin	3.552	2465885	226565	2.00	1.6	5452	2
5	Sitagliptin	2.112	759245	72584		1.7	5584	3
6	Simvastatin	3.560	2489578	221542	2.04	1.6	5456	3

$$\%\text{Assay} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity

Table No.5: Sitagliptin Linearity

S.No	Concentration g/ml	Peak Area
1	6	467849
2	8	619854
3	10	768784
4	12	928977
5	14	1095698

Table No.6: Simvastatin Linearity

S.No	Concentration g/ml	Average Peak Area
1	18	1789546
2	24	2456987
3	30	3085985
4	36	3759864
5	42	4406589

Precision

Table No.7: Results for Precision of Sitagliptin and Simvastatin

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Sitagliptin	2.108	766854	702564	5685	1.6
2	Sitagliptin	2.105	765884	698789	5584	1.4
3	Sitagliptin	2.113	765842	701235	5521	1.6
4	Sitagliptin	2.109	768985	700124	5525	1.9
5	Sitagliptin	2.109	765845	698986	5578	1.7
Mean			766682			
Std. Dev			1357.973			
% RSD			0.177123			

Table No.8: Results for Precision of Sitagliptin and Simvastatin

S.No	Name	Rt	Area	Height	USP platecount	USP Tailing
1	Simvastatin	3.552	2569865	2231111	5365	1.6
2	Simvastatin	3.550	2578474	2674210	5425	1.6
3	Simvastatin	3.564	2568985	2231261	5368	1.5
4	Simvastatin	3.564	2586845	2421301	5359	1.5
5	Simvastatin	3.565	2545898	2324710	5498	1.6
Mean			2570013			
Std. Dev			15309.45			
% RSD			0.595695			

Accuracy

Table No.9: Accuracy results for Sitagliptin

S.No	%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
1	50%	392891.7	5	5.027	100.540%	100.351%
2	100%	781996	10	10.026	100.260%	
3	150%	1171988	15	15.038	100.253%	

Table No.10: Accuracy results for Simvastatin

S.No	% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
1	50%	204962	15	15.156	101.040%	100.93%
2	100%	365018	30	30.378	101.260%	
3	150%	521064.3	45	45.218	100.484%	

Table No.11: Results of Validation Parameters

S.No	Parameter	Requirement	Result		Acceptance Criteria
			Sitagliptin	Simvastatin	
1	System Suitability	Retention time	2.10	3.53	-----
2		Tailing Factor	0.97	1.26	Should not be MT 2
3		Plate count	5587	5398	Should be MT 2000
4		Resolution	-	2.97	Should be MT 2
5	Percentage Purity	Assay value	99.7 %	100.4 %	100±2%
6	Accuracy	% Recovery	100.35%	100.93%	98-102%
7	Precision Intermediate Precision (Analyst -1.)	%RSD	0.07	0.16	NMT 2%
8	Linearity	Correlation coefficient (r^2)	0.999	0.999	Should not be LT 0.999
9	LOD	-	0.6	0.8	NMT 3
10	LOQ	-	1.80	2.40	NMT 10

Basic Instrumentation

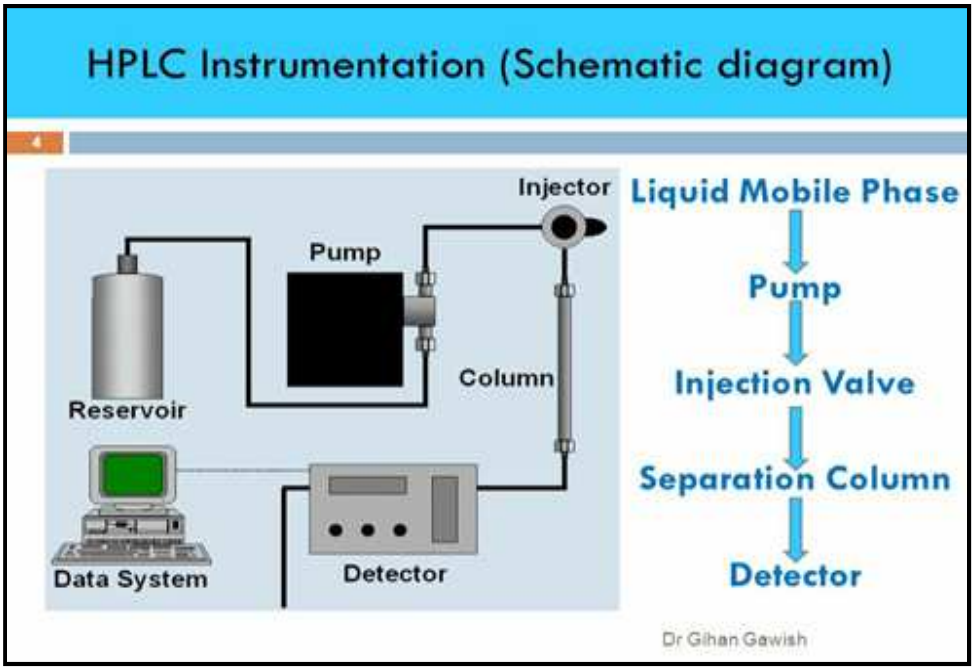

Figure No.1: HPLC Instrumentation

Method Development Trials

Optimized Trial

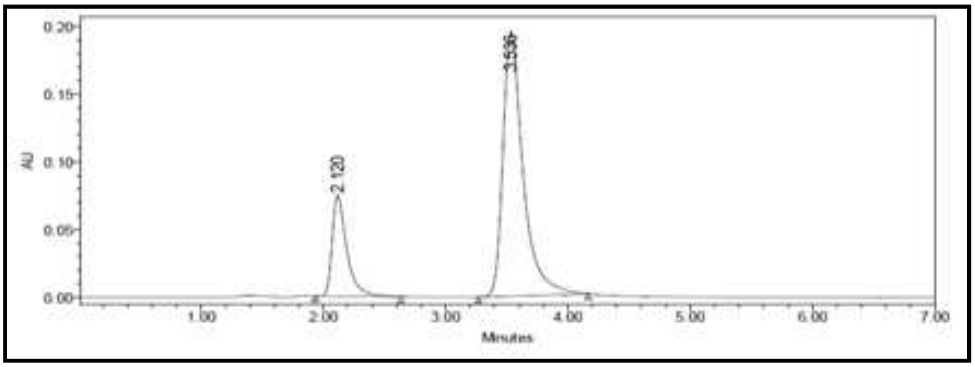

Figure No.2: Optimized Chromatogram

Validation

Assay: Standard

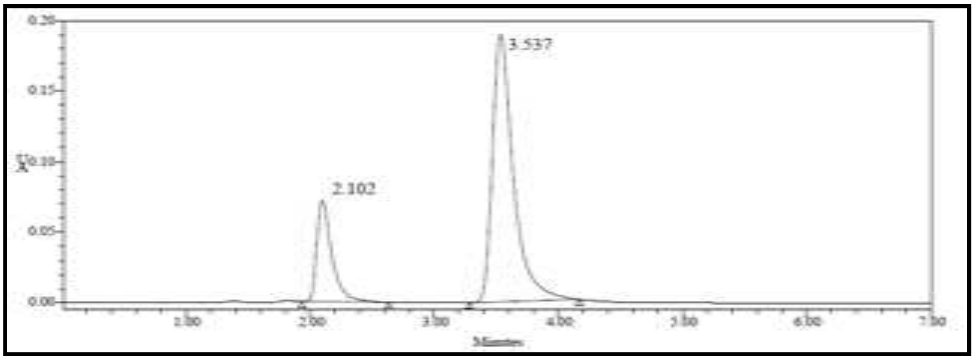

Figure No.3: Chromatograms for Assay of standard

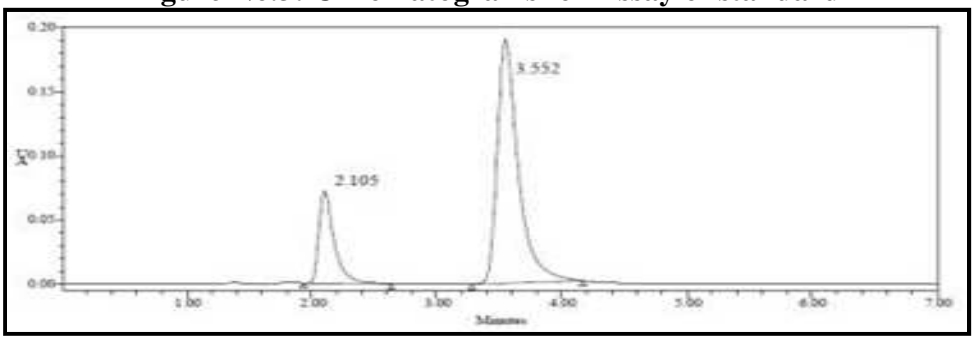

Figure No.4: Chromatograms for Assay of standard

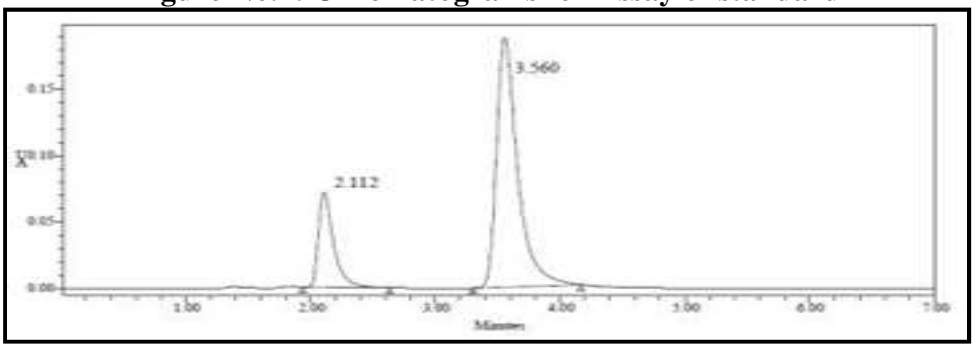

Figure No.5: Chromatograms for Assay of standard

Sample

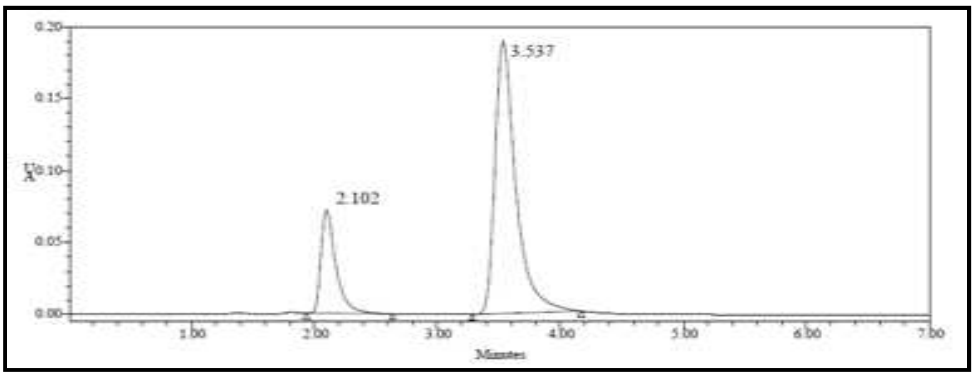

Figure No.6: Chromatogram for Assay of sample

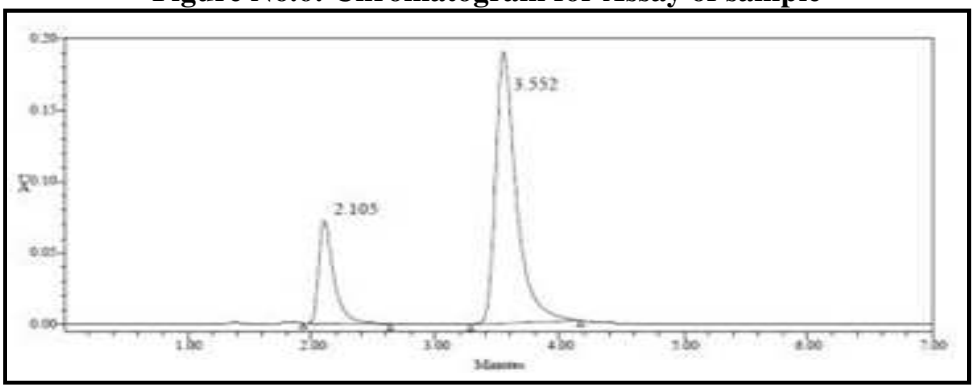

Figure No.7: Chromatogram for Assay of sample

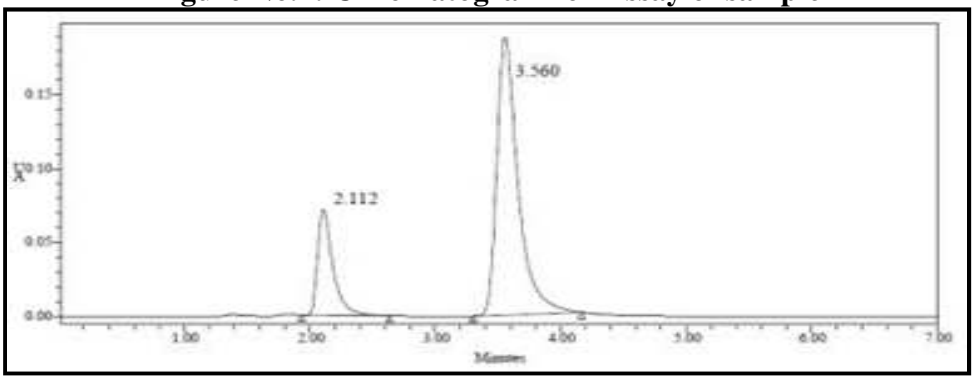

Figure No.8: Chromatogram for Assay of sample

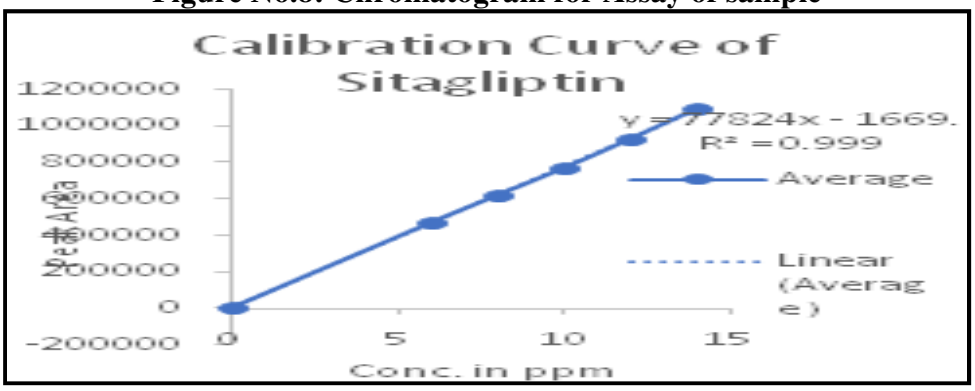

Figure No.9: Calibration curve of Sitagliptin and Simvastatin

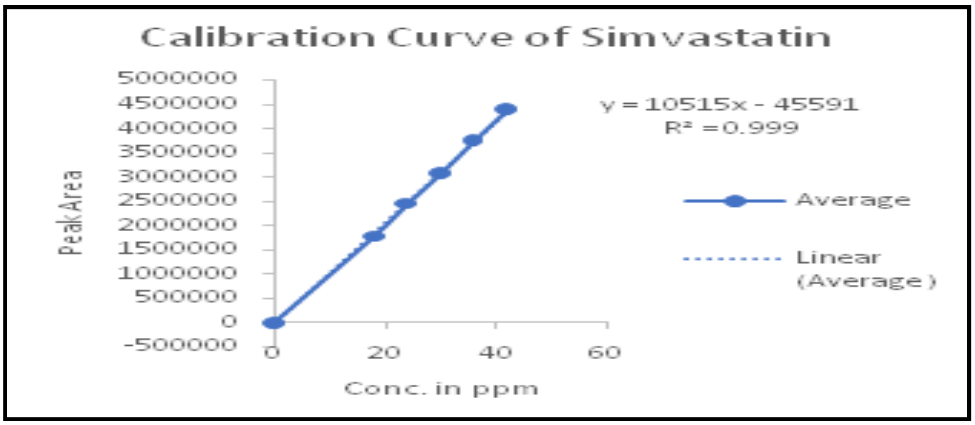

Figure No.10: Calibration curve of Sitagliptin and Simvastatin

CONCLUSION

A new method was established for simultaneous estimation of Sitagliptin and Simvastatin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Sitagliptin and Simvastatin by using Phenomenex Luna C18 (4.6×250mm, 5µm) particle size column, with a flow rate of 1ml/min, using Acetonitrile: Phosphate Buffer of pH-4.6 (adjusted with Orthophosphoric acid), in the ratio of 45:55% with a detection wave length of 245nm. The instrument used was WATERS HPLC Auto Sample r, Separation module 2695, detection was carried by using photo diode array (PDA) detector 996, with an Empower-software version-2. The retention times of Sitagliptin and Simvastatin were found to be 2.102mins and 3.537mins respectively and the % purity was found to be 99.8%. The system suitability parameters such as theoretical plates and tailing factor were found to be within limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1). By observing the correlation coefficient value as 0.999 for both the drugs at a concentration range of 6µg-14µg and 18µg-42µg respectively, we have concluded the method as linear. From the % recovery as 100.351% and 100.93% for both the drugs respectively we have concluded our method as accurate. %RSD for repeatability was found to be 0.177 and 0.595 respectively which concludes our method as repeatable and precise. LOD value was 0.6 and 0.8, and LOQ value was 1.8 and 2.4 respectively.

Hence the suggested RP-HPLC method can be used for routine analysis of Sitagliptin and Simvastatin in pure and Pharmaceutical dosage form.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Samskruti College of Pharmacy, Ghatkesar, Medchal, Hyderabad, Telangana, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Mounika R *et al*. Analytical method development and validation for quantitative determination of Sitagliptin and Simvastatin in bulk and marketed dosage form by RP-HPLC, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry,* 9(3), 2021, 124-133.