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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN AND SIMVASTATIN IN COMBINED DOSAGE FORM BY RP – UPLC METHOD

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Abstract:

The present work was aimed at developing a validated RP-UPLC method for simultaneous estimation of Sitagliptin (Anti diabetic agent) and Simvastatin (anti cholesteremic agent). This method incorporates Acetonitrile : orthophosphoric acid pH4 (70:30) as mobile phase at a flow rate of 0.4ml/min. The optimum wavelength selected was 213nm and run time was 3mins. The column used was BEH C18 with dimensions of 2.1 x 100mm and particle size of 1.7µs. The method was validated for its linearity, accuracy, precision, specificity, robustness. The system suitability parameters passed in which the asymmetric factors for sitagliptin and simvastatin were 1.5 and 1.3, respectively with linearity in the range of 500 to 900 for sitagliptin and 200 to 360ppm for simvastatin with 0.999 as the value of correlation coefficient. Accuracy studies we done and Results in terms of % recovery for sitagliptin and simvastatin was observed as 100.6%, 100.1% respectively. The faster retention times (0.502 and 1.583 mins of sitagliptin and simvastatin respectively) and better resolutions obtained by this method can be considered to be advantageous Thus a novel, validated and sensitive RP-UPLC method was developed for simultaneous estimation of sitagliptin and simvastatin in combined dosage form.

Key words: RP-UPLC, System suitability parameters, Linearity, Accuracy.

Introduction:

Sitagliptin a very selective DPP-4 inhibitor with Molecular formula $C_{16}H_{15}F_6N_5O{\bullet}H_3PO_4{\bullet}H_2O$ and IUPAC name 7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]- 5, 6, 7, 8-tetrahydro-3-(trifluoromethyl)-1, 2,4- Triazolo [4,3a] pyrazine phosphate is believed to exert its actions in type 2 diabetes patients by slowing the inactivation of incretin hormones, thereby increasing concentration and prolonging the action of these hormones. By increasing and prolonging

active incretin levels, sitagliptin increases insulin release and decreases glucagon levels in blood circulation in a glucose-dependent manner. Sitagliptin demonstrates selectivity for DPP-4 and does not inhibit DPP-8 or DPP-9 activity in vitro at concentrations approximating those from therapeutic doses.[1, 2] Simvastatin on the other hand is anti cholesteremic agent that inhibits an early and rate-limiting step in cholesterol biosynthesis i.e., the conversion of HMG-CoA to mevalonic acid by blocking HMG-CoA reductase. Thereby Simvastatin reduces total cholesterol, LDL-cholesterol and triglycerides and increases HDL-cholesterol levels[3]. The molecular formula and chemical name of Simvastatin are $C_{25}H_{38}O_5$(1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl- 2,2-dimethylbutanoate respectively. Review of literature revealed that there are only HPLC methods reported for the estimation of Sitagliptin and Simvastatin in combined dosage form. To date there are no validated RP-UPLC methods published with faster retention times and better resolution.

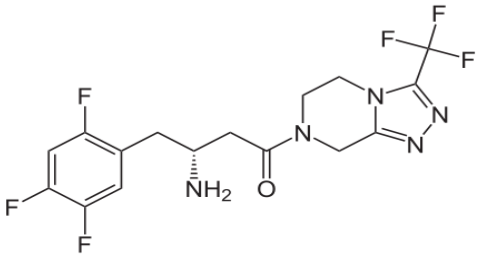


Fig. 1: Structure of Sitagliptin.

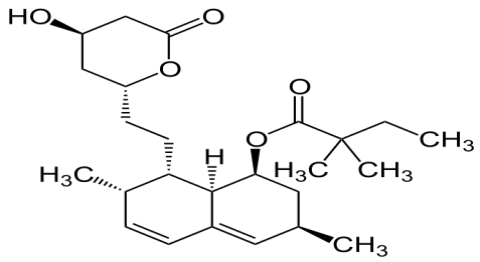


Fig. 2: Structure of Simvastatin.

Materials and Methods

Instrumentation: Water's Aquity UPLC with Empower software

BEH C18 Column with dimensions of 2.1 x 100mm and particle size of 1.7µs

Chemicals and reagents

Analytically pure Sitagliptin and Simvastatin were provided by MSN laboratories, Hyderabad as a gift sample. Water, acetonitrile and all other chemicals of UPLC analytical grade were purchased from Thermo Fisher Scientific India Pvt. Ltd, Mumbai.

Chromatographic conditions[4, 5]

Glassware used in each procedure were soaked overnight in a mixture of chromic acid and sulfuric acid, rinsed thoroughly with double distilled water and dried in hot air oven. Acetonitrile and orhophosphoric acid pH 4 were used in ratio of 70:30 as mobile phase. The contents of the mobile phase were filtered before use through a 0.45µ membrane and degassed for 20 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.4 ml/min and injection volume was 20µL. The column temperature was maintained at ambient temperature. The eluents were monitored at 213nm using UV lamp.

Preparation of Ortho phosphoric acid (pH4):

Weighed 7.0grams of sodium dihydrogen ortho phosphate and transferred it into a 1000ml beaker, this was dissolved and diluted to 1000ml with UPLC grade water. Finally the pH was adjusted to 4 with Triethylamine.

Preparation of mobile phase:

Mixed a mixture of above buffer 300 mL (30%) and 700 mL of Acetonitrile UPLC (70%) and degased it in ultrasonic water bath for 5 minutes. Final solution was filtered through 0.45 µ filter under vacuum filtration.

The same mobile phase was used as diluent

Preparation of the Sitagliptin& Simvastatin Standard & Sample Solution:

Standard Solution Preparation:

Accurately weighed and transferred 100 mg & 40 mg of Sitagliptin and Simvastatin working standard into a 100mL clean dry volumetric flask. Then about 70mL of Diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. (Stock solution)

Further pipetted 7ml of Sitagliptin& Simvastatin from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Sample Solution Preparation:

Accurately weighed and transferred 208.9 mg of Sitagliptin and Simvastatin Tablet powder into a 100mL clean dry volumetric flask then added about 70mL of diluent, sonicated to dissolve it completely and volume was made up to the mark with the same solvent (Stock solution). Further pipetted 7ml of Sitagliptin& Simvastatin from the above stock solution into a10mL volumetric flask and diluted up to the mark with diluent.

Estimation Method: 20 µL of the standards and sample were injected seperately into the chromatographic system and the areas for the Sitagliptin and Simvastatin peaks were measured and the %Assay was calculated using the formulae.

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt.}}{\text{Label Claim}} \times 100$$

Where:

AT= average area counts of sample preparation.

AS = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = LABEL CLAIM OF DRUG mg/ml.

Results and Discussion

After several trails with various solvents, mobile phase system composed of phosphate buffer of P^H 4.0 and acetonitrile in the proportion of 30:70 respectively was chosen for the simultaneous estimation and validation of sitagliptin and simvastatin in combined dosage form by RP-UPLC. This mobile phase composition offered maximum resolution for the drug at the detection wavelength of 213nm. Mobile phase with the flow rate of 0.4 ml/min gave optimum separation with good resolution between the peaks. A reverse phase BEH column was used as stationary phase. The retention time of sitagliptin and simvastatin were found to be 0.503 and 1.613 minutes, respectively. The total time of analysis was less than 3 minutes. The percentage purity for sitagliptin and simvastatin were found to be 99.7 and 99.1, respectively.

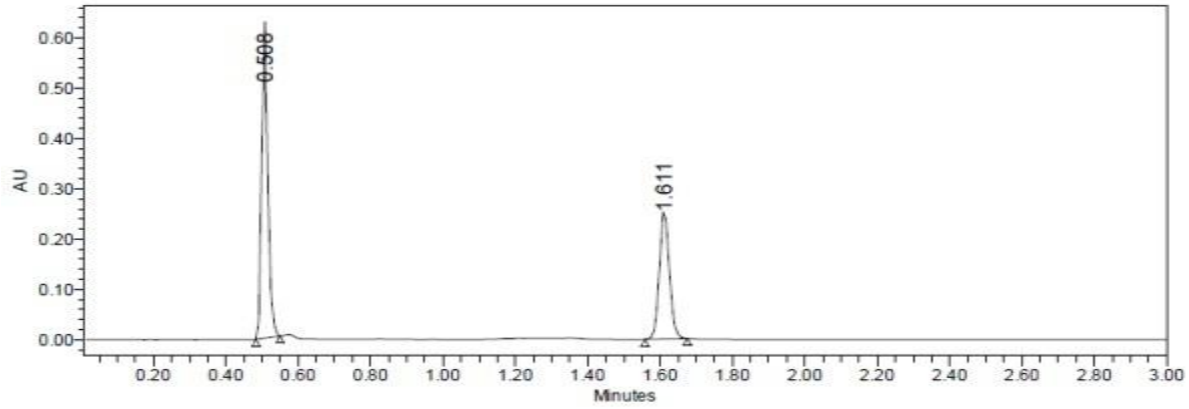


Fig 3: Chromatogram of Sitagliptin and Simvastatin Formulation.

Method Validation parameters[6, 7]:

Method was validated as per ICH guidelines with respect to linearity, accuracy, precision, specificity, and robustness, limit of detection and limit of quantification.

Linearity: From the calibration curve constructed by plotting concentration vs. peak area, it was found that there exists a linear relationship in the concentration range of 500 to 900 for sitagliptin, with 0.999 as the value of correlation coefficientand for simvastatin the linearity in the range of 200 to 360ppm with 0.999 as the value of correlation coefficient.

Table 1: Linearity Results: (for Sitagliptin)

S.No	Linearity Level	Concentration	Area
1	I	500ppm	521793
2	II	600ppm	620803
3	III	700ppm	713828
4	IV	800ppm	827261
5	V	900ppm	932646
Correlation Coefficient			0.999

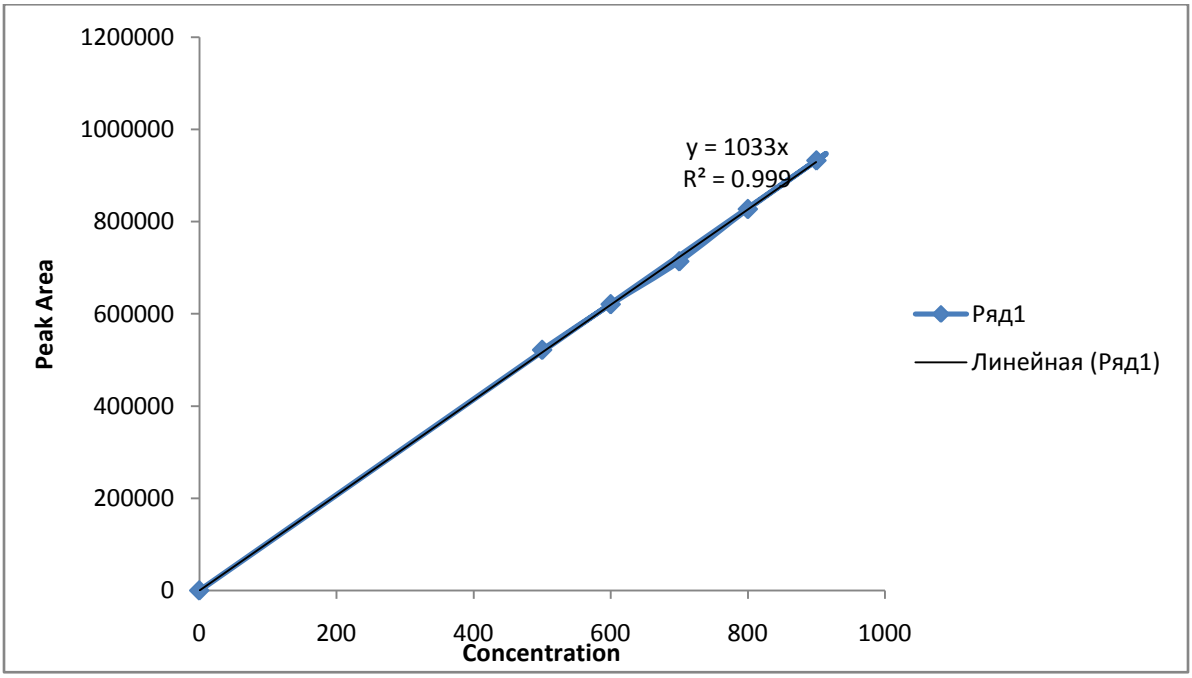


Fig.4: Linearity curve of Sitagliptin.

Table 2: Linearity Results (for Simvastatin)

S.No	Linearity Level	Concentration	Area
1	I	200ppm	399013
2	II	240ppm	464022
3	III	280ppm	529213
4	IV	320ppm	596276
5	V	360ppm	656691
Correlation Coefficient			0.999

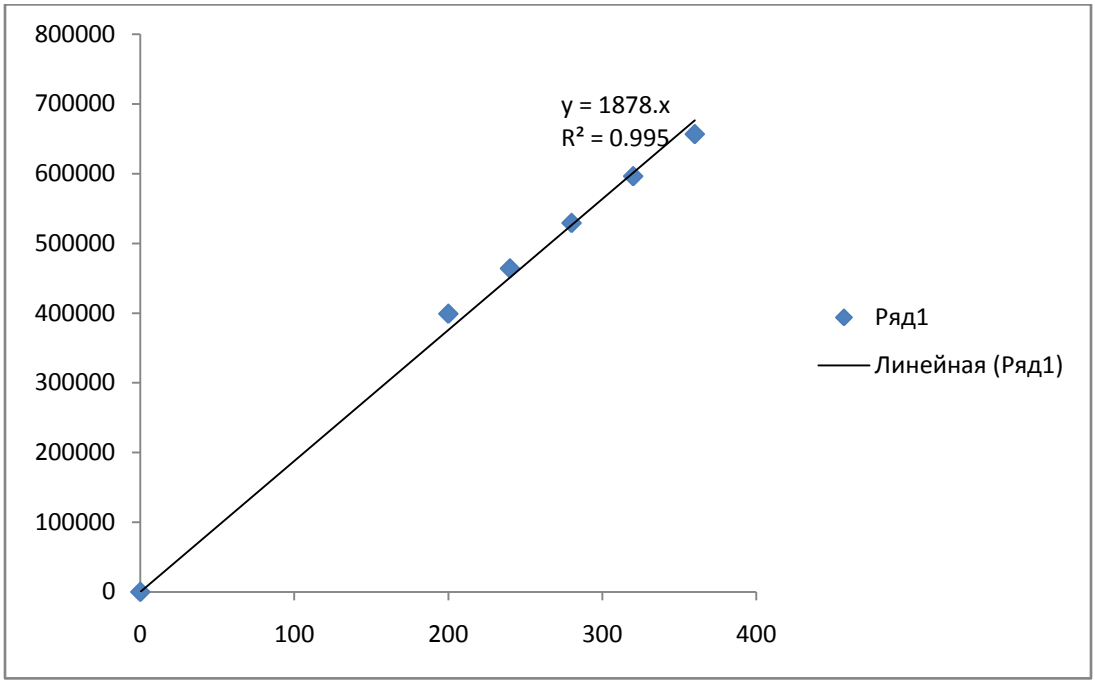


Fig.5: Linearity curve of Simvastatin.

System suitability: System suitability studies were carried out in which the asymmetric factors for sitagliptin and simvastatin were 1.5 and 1.3, respectively. Sitagliptin was found to have a value of 2556.4 as its number of Theoretical plates and for Simvastatin it was 23185.

Precision:

For method precision, the sample solution at working concentration was analyzed in replicate as per the assay method. The percentage relative standard deviation was calculated for the peak areas of each drug and it was found to be 0.390062 for Sitagliptin and 0.954836 for Simvastatin.

Table 3: The results of system suitability (Sitagliptin)

Injection	**Area**
Injection-1	**746921**
Injection-2	**745146**
Injection-3	**747076**
Injection-4	**747336**
Injection-5	**740412**
Average	**745378.2**
Standard Deviation	**2907.439**
%RSD	**0.390062**

Table 3: The results of system suitability (Simvastatin):

Injection	**Area**
Injection-1	**519174**
Injection-2	**510022**
Injection-3	**511778**
Injection-4	**512311**
Injection-5	**521078**
Average	**514872.6**
Standard Deviation	**4916.19**
%RSD	**0.954836**

Accuracy: The accuracy of the method was studied by performing recovery studies at 50%, 100% and 150% level. The standard drug at the concentration level of 50%, 100% and 150% were added to the sample and the analysis was carried out as per the assay method. The results were expressed in terms of percentage recovery. The values were found to be 100.6% at 50%, 99.6% at 100% level, 99.3% at 150% level and 98.4% at 50% level, 99.7% at 100% level, 100.1% at 150% level for sitagliptin and simvastatin, respectively

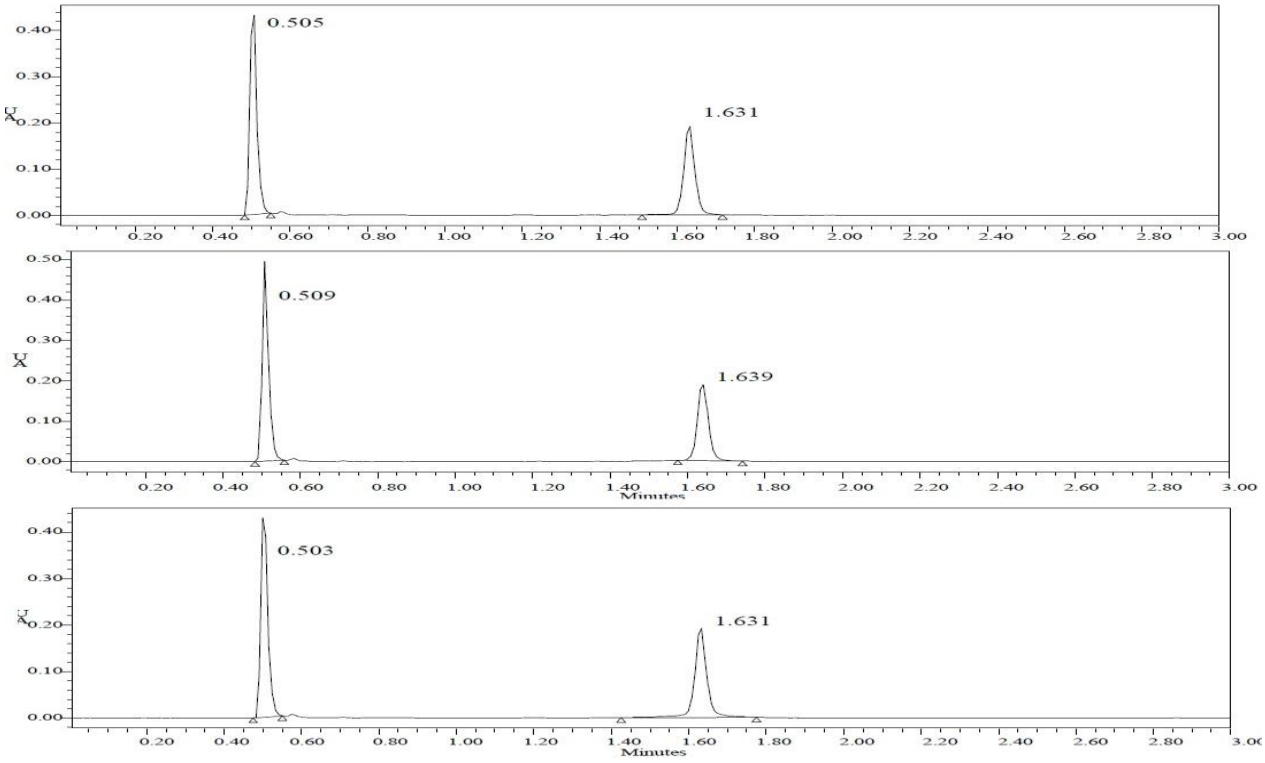


Fig 6: Chromatogram of Recovery Studies at 50% Level.

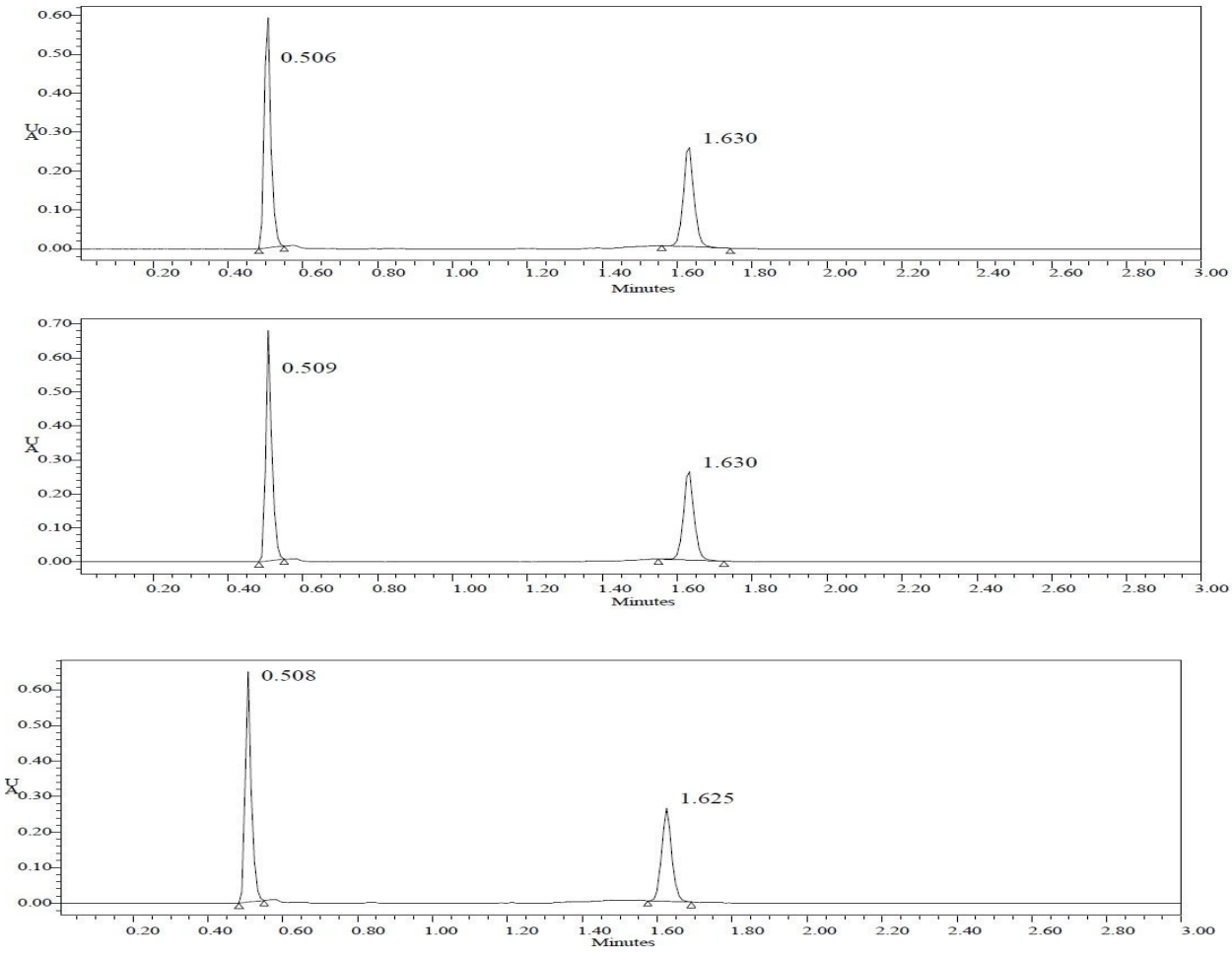


Fig.7: Chromatogram of Recovery Studies at 100% Level.

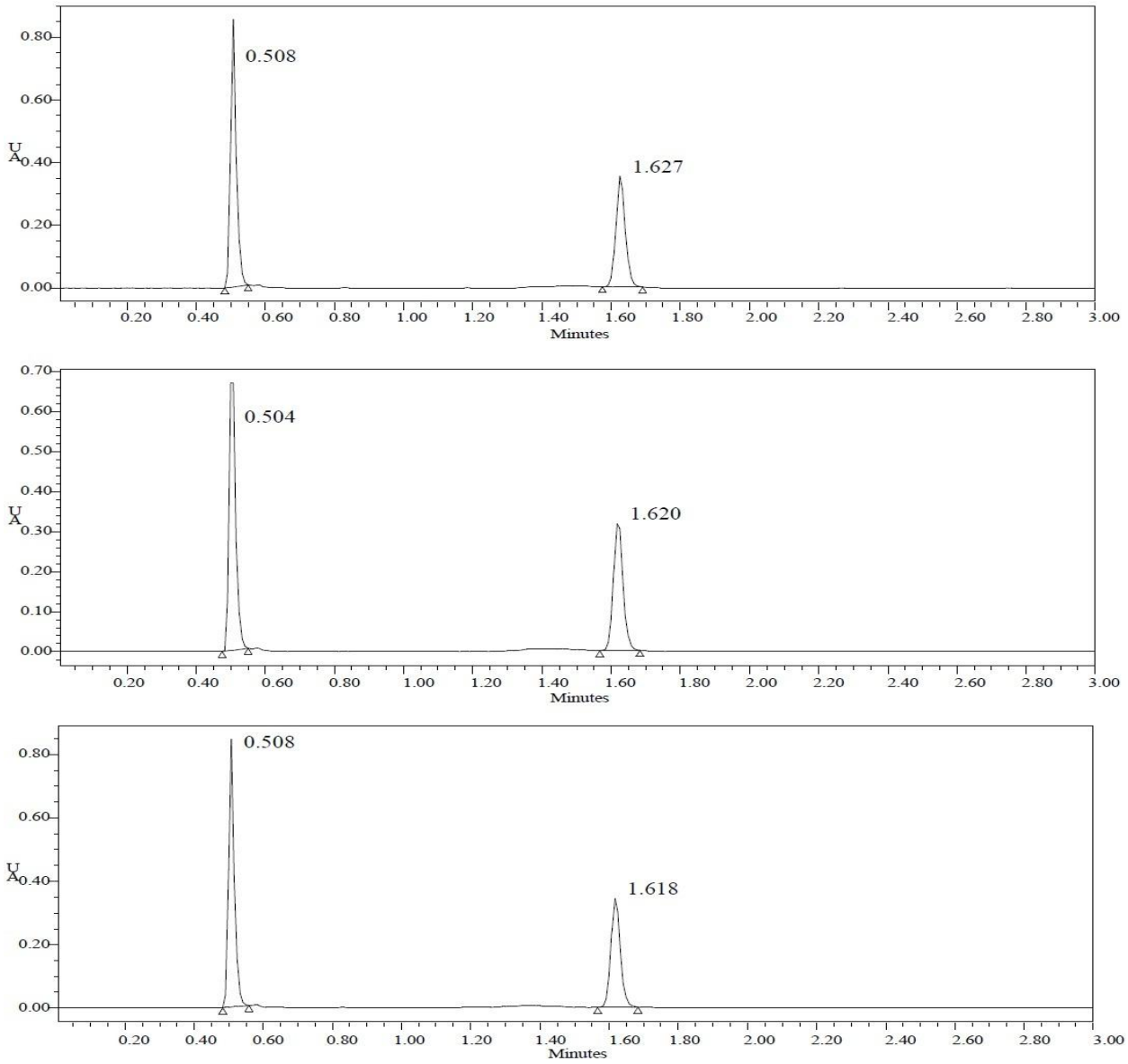


Fig.8: Chromatogram of Recovery Studies at 150% Level.

Table 5: The accuracy results for Sitagliptin.

%Concentration (at specification Level)	**Area**	**Amount Added (mg)**	**Amount Found (mg)**	**% Recovery**	**Mean Recovery**
50%	376680	50	50.3	100.6%	99.8%
100%	746110	100	99.6	99.6%	
150%	1116092	150	149.0	99.3%	

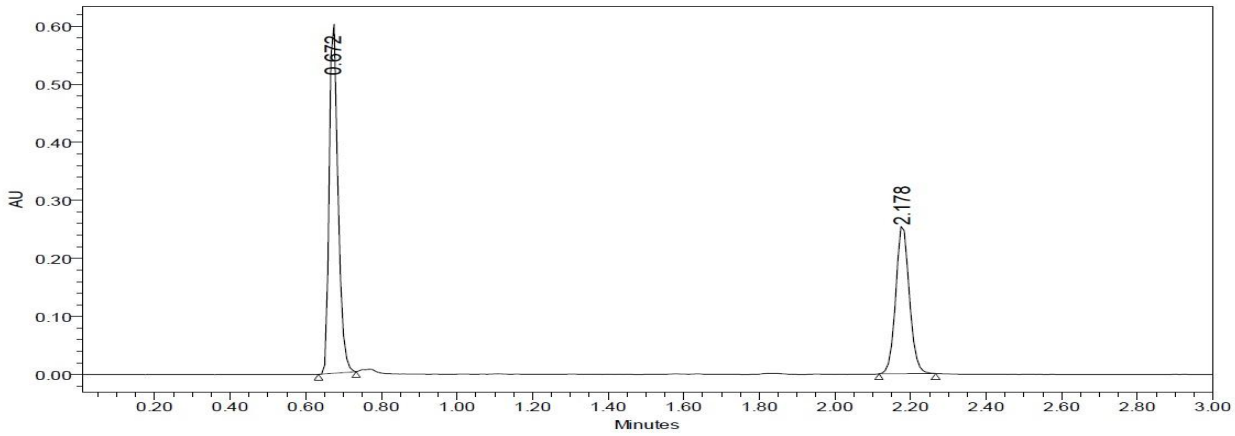
Table 6:The accuracy results for Simvastatin.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	253868	20	19.6	98.4%	99.4%
100%	514331	40	39.9	99.7%	
150%	774118	60	60.0	100.1%	

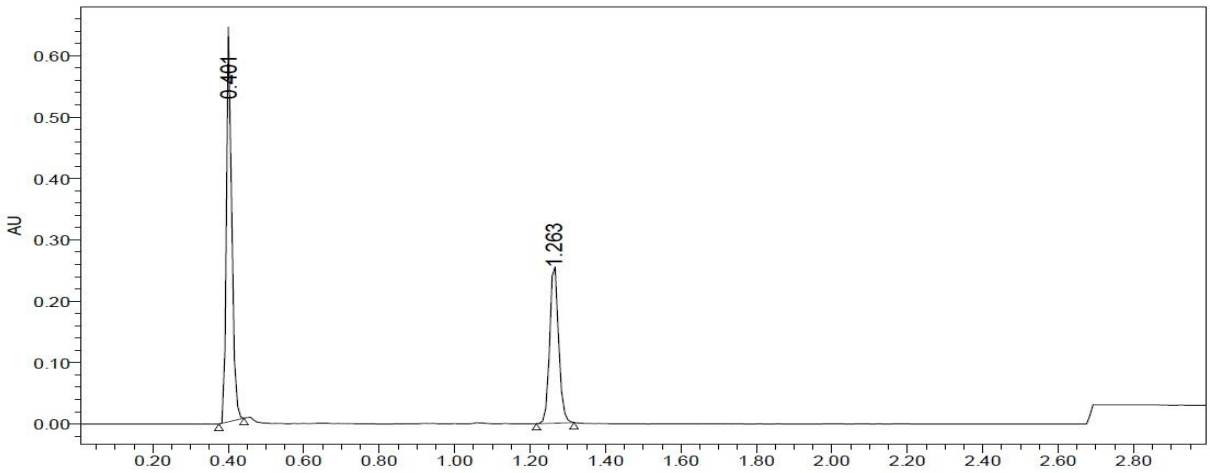
Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Variation were made to evaluate the impact on the method.

Robustness

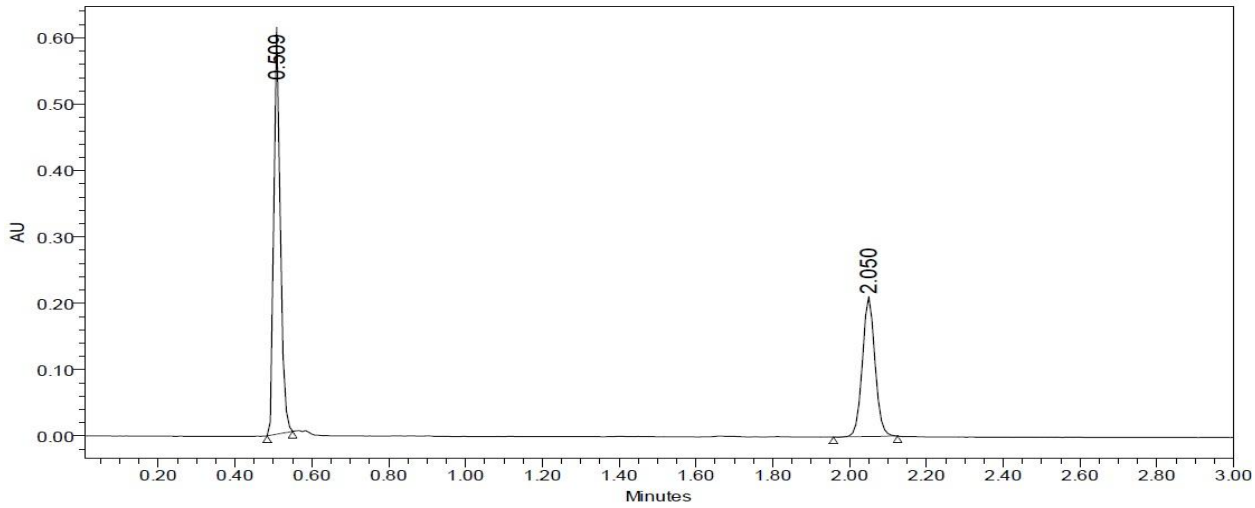


Less flow rate

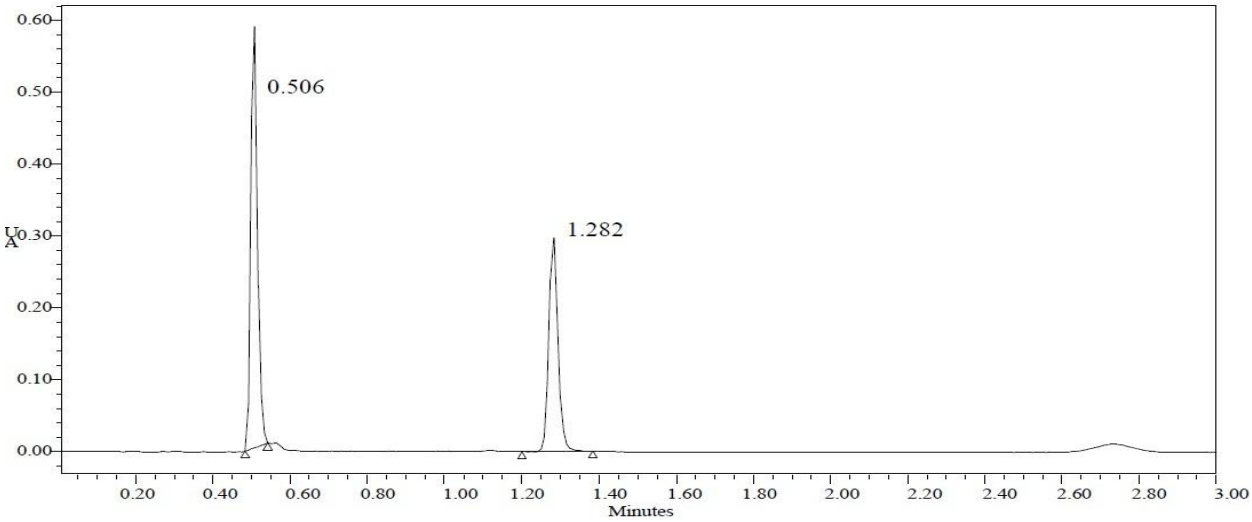


More flow rate

Fig.9: Change in flow rate



Less portion of organic phase



High portion of organic phase

Fig.10: Change in mobile phase composition.

Table 7: Change in flow rate Robustness (Sitagliptin)

No	**Flow Rate (ml/min)**		
		USP Plate Count	**SP Tailing**
	0.3	3178.8	1.3
	4	2556.4	1.3
	0.5	2118.2	1.2

Table 8: Change in flow rate Robustness (Simvastatin)

No	**Flow Rate (ml/min)**		
		USP Plate Count	**SP Tailing**
	0.3	15759.1	1.1
	0.4	23185.6	1.1
	1.5	11131.1	1.1

Table 9: Change in mobile phase composition Robustness (Sitagliptin)

No	Change in Organic Composition in the Mobile Phase	ystem Suitability Results	
		USP Plate Count	**SP Tailing**
	10% less	2487.3	1.2
	Actual	2556.4	1.3
	10% more	2341.5	1.1

Table 10: Change in mobile phase composition Robustness (Simvastatin)

No	**Change in Organic Composition in the Mobile Phase**	**ystem Suitability Results**	
		USP Plate Count	**SP Tailing**
	10% less	16227.7	1.0
	Actual	23185.6	1.1
	10% more	11186.3	1.1

LOD & LOC: Limit of measurements such as limit of detection and limit of quantification were found to be 0.025µg and 0.083µg for sitagliptin and For Simvastatin 0.105µg and 0.305µg, respectively.

Table 11 LOD and LOQ Values

Sample	**Limit of Detection (µg)**	**Limit of Quantification (µg)**
SIT	0.025	0.083
SIM	0.105	0.350

Table 12: Summary of the Method Developed

Parameter	**Observation**	
	SIT	**SIM**
Label claim (mg/tab)	100	40
% Label claim	99.7	99.1
Linearity range (µg/ml)	500 to 900	200 to 360
Correlation coefficient (NLT 0.999)	0.999	0.999
Asymmetry factor (NMT 2%)	1.5	1.3
Number of Theoretical Plates (NLT 2000)	2556.4	23185
precision % RSD (NMT 2%)	0.390062	0.954836
%Recovery (98 to 102%)	99.7	99.1
Limit of Detection (µg)	0.025	0.105
Limit of Quantification **(**µg)	0.083	0.350

Conclusion:

The proposed RP-UPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Sitagliptin and Simvastatin in combined tablet dosage form. This method was validated as per ICH guidelines. 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, this method can be easily and conveniently adopted for routine analysis of Sitagliptin and Simvastatin in combined tablet dosage form.

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