International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.3, pp 880-893, July-Aug 2014

A New, Simple, Sensitive, Accurate & Rapid Analytical Method Development & Validation for Simultaneous Estimation of Sitagliptin & Simvastatin in Tablet dosage form by using UPLC

G. Sravan Kumar Reddy*[1], S. Ashutosh Kumar[2], V. Raj Kumar[3]

[1]Department of Pharmaceutical Analysis & Quality Assurance, Sana College of Pharmacy, Kodad, Nalgonda, A.P, India
[2]Department of Pharmaceutical Analysis & Quality Assurance, A.K.R.G College of Pharmacy, Nallajerla, West Godavari, 534112, A.P, India.
[3]Department of Pharmaceutical Analysis & Quality Assurance, Pratista Institute of Pharmaceutical Sciences, Suryapeta, Nalgonda, A.P, India

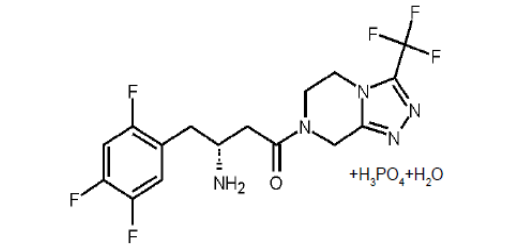
***Corres. Author: sravan.pharma@gmail.com, ashu.mpharm2007@gmail.com, Mobile No: +919963985925**

Abstract: The present work was undertaken with the aim to develop and validate a rapid and consistent UPLC method in which the peaks will be appear with short period of time as per ICH Guidelines. The proposed method was simple, fast, accurate and precise method for the Quantification of drug in the dosage form, bulk drug as well as for routine analysis in Quality control. UPLC method was developed and validated for simultaneous estimation of Sitagliptin and Simvastatin in bulk drug and in combined dosage forms. UPLC separation was achieved on a Symmetry C_{18} (2.1 x 100mm, 1.7µm, Make: BEH) or equivalent under an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (30%) whose pH was adjusted to 4.0 by using TEA & Acetonitrile (70%)[UPLC Grade]. The flow rate was monitored at 0.4 ml per min. The wavelength was selected for the detection was 213 nm. The run time was 3min. The retention time found for the drugs Sitagliptin and Simvastatin were 0.509 min. & 1.623 min. respectively. The linearity was established in the range of 500 to 900ppm for the drug Sitagliptin & 200 to360ppm for the drug Simvastatin. The LOD for the drugs Sitagliptin & Simvastatin were found to be 0.18µg/ml & 0.17µg/ml respectively. The LOQ for the drugs Sitagliptin & Simvastatin were found to be 0.61µg/ml & 0.56µg/ml respectively. The proposed method was adequate sensitive, reproducible, and specific for the determination of Sitagliptin and Simvastatin in bulk as well as in Tablet dosage form. The validation of method was carried out utilizing ICH-guidelines. The described UPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form. Overall the proposed method was found to be suitable and accurate for the Quantitative determination of the drug in Tablet dosage form. The method was simple, precise, accurate and sensitive and applicable for the simultaneous determination of Sitagliptin and Simvastatin in bulk drug and in combined dosage forms.
Keywords: Sitagliptin, Sitagliptin, ICH Guideline, UPLC, LOD, LOQ.

Introduction

Sitagliptin phosphate monohydrate (SPM)chemically, (3*R*)-3-amino-1-[3-(trifluoromethyl)-5,6-Dihydro [1,2,4] triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)butan-1-one phosphate hydrate (Fig. No.1) is

oral hypo glycemic drug of the dipeptidyl peptidase-4(DPP-40 inhibitor class. DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes that functions to stimulate glucose dependent insulin release and reduce glucagons levels. This is done through inhibition of the inactivation of in cretins, particularly glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycemic control1-3. Several analytical methods based on UV[1-6], spectroflourimetry[6], RP-HPLC[7-8], LC-MS/MS[9-11] was reported for the determination of Sitagliptin phosphate monohydrate in plasma and urine of humans, rats and dogs. Simvastatin (SIM), a methylated analog of lovastatin, is -(+)-{1S,3R,7S,8S,8aR)-1,2,3,7,8,8 a-hexahydro-3,7-dimethyl-8-[2-(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]-naphthyl-2,2-dimethyl butanoate (Fig. No.2). It acts by inhibiting HMG CoA reductase and is used for the treatment of hypercholesterolemia. After oral administration, this prodrug is converted into β- hydroxy acid of simvastatin, which is a potent inhibitor of HMG CoA reductase, a key enzyme required for the synthesis of cholesterol in liver. The determination of Simvastatin has been carried out in tablets by UV-Spectrophotometry[12- 14], RP-HPLC[15-20]. A literature review reveals that no UPLC analytical method is available for the simultaneous estimation of Sitagliptin and Simvastatin in tablet dosage form in pharmaceutical preparations, which prompted to pursue the present work. The objective of the present work is to develop and validate new analytical methods for simultaneous determination of Sitagliptin and Simvastatin in tablet dosage form. This communication forms the first report of a simple, sensitive and reproducible method for the simultaneous estimation of Sitagliptin and Simvastatin from combined dosage form by UPLC. Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows about 9-fold decreases in analysis time as compared to the conventional HPLC system using 5 µm particle size analytical columns, and about 3-fold decrease in analysis time in comparison with 3 µm particle size analytical columns without compromise on overall separation.



(3*R*)-3-amino-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a] pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)butan-1-one

Sitagliptin

Fig. no. 1-Chemical Structure of Sitagliptin

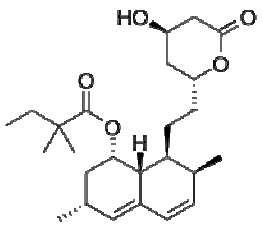


Fig. no. 2-Chemical Structure of Simvastatin

Materials & Method [21-22]

Chemicals and Reagents Used:

The following chemicals were procured for the process: Water [UPLC Grade], Methanol [UPLC Grade], Acetonitrile [UPLC Grade], Sitagliptin and Simvastatin [Working standards], Orthophosphoric Acid & Sodium Dihydrogen Ortho Phosphate all the chemicals were procured from STANDARD SOLUTIONS and the tablets were collected from the Local market.

Apparatus and Chromatographic Conditions:

Equipment: Ultra performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.

UV/VIS spectrophotometer: LAB INDIA UV 3000$^+$

pH meter: Adwa – AD 1020

Weighing machine: Afcoset ER-200A

Temperature: Ambient

Column: Symmetry C_{18} (2.1 x 100mm, 1.7µm, Make: BEH) or equivalent

Phosphate Buffer: 7.0 grams of Sodium Dihydrogen Ortho Phosphate in 1000 ml Water [HPLC Grade] pH adjusted with TEA.

pH: 4.0

Mobile phase: Phosphate Buffer: Acetonitrile (30: 70v/v)

Flow rate: 0.4 ml per min

Wavelength: 213 nm

Injection volume: 0.4 µl

Run time: 3min.

Preparation of Phosphate buffer [23]: The buffer solution was prepared by dissolving accurately weighed 7.0 grams of Sodium Dihydrogen Ortho Phosphate and transferred into a clean and dry 1000ml volumetric flask, dissolved and diluted with 1000ml water [UPLC Grade]. The final pH of the buffer was adjusted to 4.0 by using TEA.

Preparation of mobile phase: The Mobile Phase was prepared by mixing 300 ml (30%) of the above buffer and 700 ml of Acetonitrile [UPLC Grade] (70%) and degassed in an ultrasonic water bath for 10 minutes. Then the resultant solution was filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as Diluent.

Preparation of the Sitagliptin and Simvastatin Standard & Sample Solution:

Preparation of Stock solution: The stock solution was prepared by weighing accurately 100mg Sitagliptin and 40 mg Simvastatin and transferred into a clean and dry 100 ml volumetric flask. About 70 ml of diluent was added and sonicated. The volume was made upto the mark with the same diluent. From the above prepared Stock solution pipette out 7.0 ml of solution and transferred into a clean and dry 10ml volumetric flask, the diluent was added upto the mark to get final concentration.

Preparation of Sample Solution: The sample solution was prepared by weighing equivalently 208.9 mg of Sitagliptin and Simvastatin and transferred into a 100 ml clean and dry volumetric flask and about 70ml of diluent was added and sonicated to dissolve it completely and the volume made up to the mark with the same solvent. From above prepared stock solution pipette out 7ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added upto the mark 10ml to get final concentration. The standard and sample solutions were injected five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

System Suitability[24-26]: The Tailing factor for the peaks due to Sitagliptin and Simvastatin in Standard solution should not be more than 1.5. The Theoretical plates for the Sitagliptin and Simvastatin peaks in Standard solution should not be less than 2000. The system suitability of the method was checked by injecting five different preparations of the Sitagliptin and Simvastatin standard. The parameters of system suitability were checked.

Assay calculation for Sitagliptin & Famotidine:

Assay % = $\frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{P}{100} * \frac{Avg.Wt.}{Label\ Claim} * 100$

Where,

AT = average area counts of sample preparation.

AS = average area counts of standard preparation.

WS= Weight of working standard taken in mg.

WT=Weight of test taken in mg.

DS =Dilution of standard solution

DT =Dilution of sample solution

P = Percentage purity of working standard

System Suitability Results for Sitagliptin:

1) The Tailing factor obtained from the standard injection was **1.3.**

2) The Theoretical Plates obtained from the standard injection was **2556.4.**

Assay Result for Sitagliptin:

$$\frac{744663}{745736}\times\frac{100}{100}\times\frac{1}{1}\times\frac{7}{10}\times\frac{100}{208.9}\times\frac{1}{1}\times\frac{10}{7}\times\frac{99.8}{100}\times\frac{208.9}{100}\times 100 = 99.7\ \%$$

System Suitability Results for Simvastatin:

1) The Tailing factor obtained from the standard injection was **1.1.**

2) The Theoretical Plates obtained from the standard injection was **2318.5.**

Assay Results for Simvastatin:

$$\frac{500877}{504694}\times\frac{100}{100}\times\frac{1}{1}\times\frac{7}{10}\times\frac{100}{208.9}\times\frac{1}{1}\times\frac{10}{7}\times\frac{99.8}{100}\times\frac{208.9}{40}\times 100 = 99.1\ \%$$

Validation Development [27-34]

Precision: It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The chromatogram was represented in fig. no.6. (Table no. 1 & 2).

Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for five times and measured the area for all five injections in UPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The chromatogram was represented in fig no. 7. (Table no. 3 & 4).

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. The standard solution with Accuracy -50%, Accuracy -100% and Accuracy -150% were injected into chromatographic system and calculated the amount found and amount added for Sitagliptin & Simvastatin and further calculated the individual recovery and mean recovery values. The chromatograms were represented in fig. no. 8, 9 & 10. (Table no. 5 & 6).

Linearity: It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope or regression line. It is determined by series of three to six injections of five of more standards. Different levels of solution were prepared and injected to the chromatographic system and the peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The calibration curve was represented in fig. no. 11 & 12. (Table no. 7 & 8).

Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value.

Limit of Detection for the drugs Sitagliptin & Simvastatin: The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. Limit of detection is the lowest concentration of the substance that can be detected, not necessarily quantified by the method. (Regression statistics) The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the following formula.

Limit of detection (LOD) = $\frac{\sigma}{S}\times$ 3.3

Where S – slope of the calibration curve

σ – Residual standard deviation

Calculation of S/N Ratio for Sitagliptin:

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOD solution (0.26% of target assay concentration) : 151 µV

S/N = 151/52 = 2.96

Calculation of S/N Ratio for Simvastatin:

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOD solution (0.62% of target assay concentration) : 154 µV

S/N = 154/52 = 3.01

Acceptance Criteria: The S/N Ratio value should be 3 for LOD solution.

Limit of Quantification: It is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. LOQ is expressed as a concentration at a specified signal to noise ratio.

Limit of Quantification for the drugs Sitagliptin & Simvastatin: The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. Limit of Quantification is the lowest concentration of the substance that can be estimated quantitatively. It can be determined from linearity curve by applying the following formula

$$\text{Limit of Quantification (LOQ)} = \frac{\sigma}{S} \times 10$$

Where S – slope of the calibration curve

σ – Residual standard deviation

Calculation of S/N Ratio for Sitagliptin:

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOD solution (0.62% of target assay concentration) : 514 µV

S/N = 514/52 = 10.0

Calculation of S/N Ratio for Simvastatin:

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOQ solution (2.0% of target assay concentration) : 509µV

S/N = 509/52= 9.98

Acceptance Criteria: The S/N Ratio value should be 10 for LOQ solution.

The chromatograms were represented in fig. no. 13& 14.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The standard and samples of Sitagliptin and Simvastatin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

The flow rate was varied at 0.3 ml/min to 0.5ml/min.: The Standard solution of Sitagliptin & Simvastatin was prepared and analysed using the varied flow rates along with method developed flow rate. On evaluation of the above results, it was concluded that the variation in flow rate does not affected the method significantly. Hence it was indicated that the method was robust even by change in the flow rate. The chromatograms were represented in fig. no. 15 & 16. (Table No. 9 & 10).

The Organic composition in the Mobile phase was varied from 60% to 80%.: The Standard solution for the drug Sitagliptin & Simvastatin was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition. On evaluation of the above results, it was concluded that the variation in 10% Organic composition in the mobile phase does not affected the method significantly. Hence it was indicated that the method was robust even by change in the Mobile phase ±10. The chromatograms were represented in Fig. no. 17 & 18. (Table no. 11 & 12)

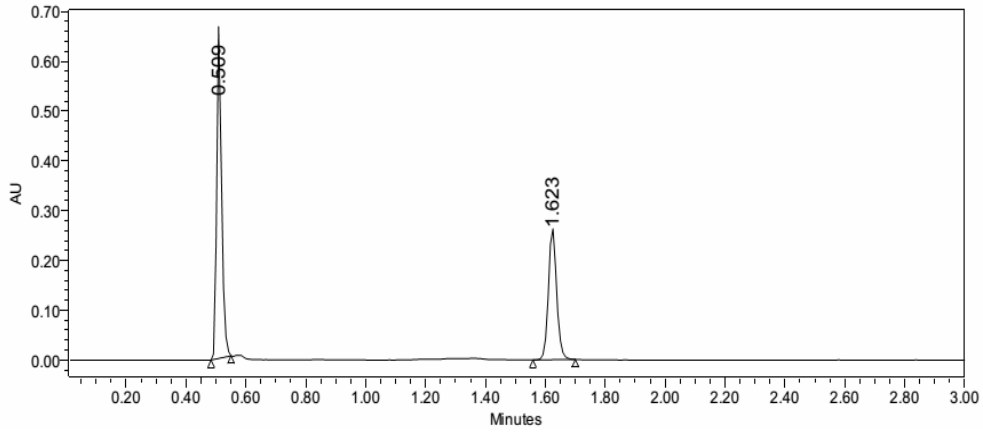


Fig. no. 4- Chromatogram for the Optimized Method Development

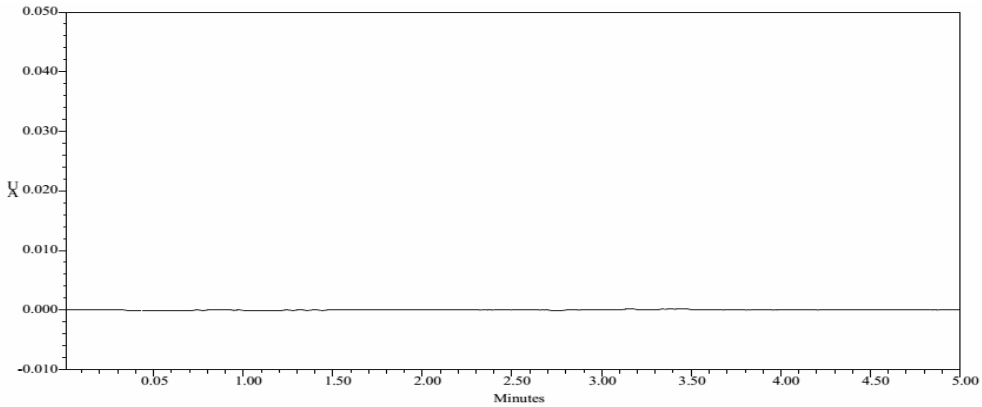


Fig. no. 5 -Chromatogram for the Blank

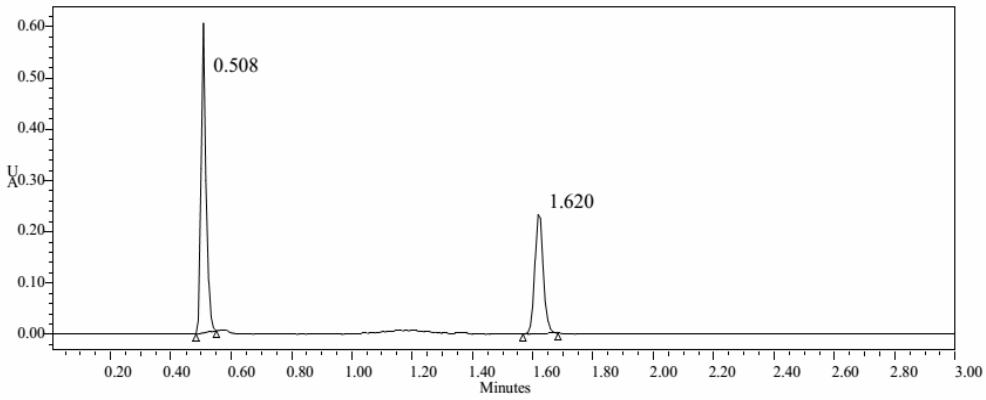


Fig. no.6- Chromatogram for the Precision

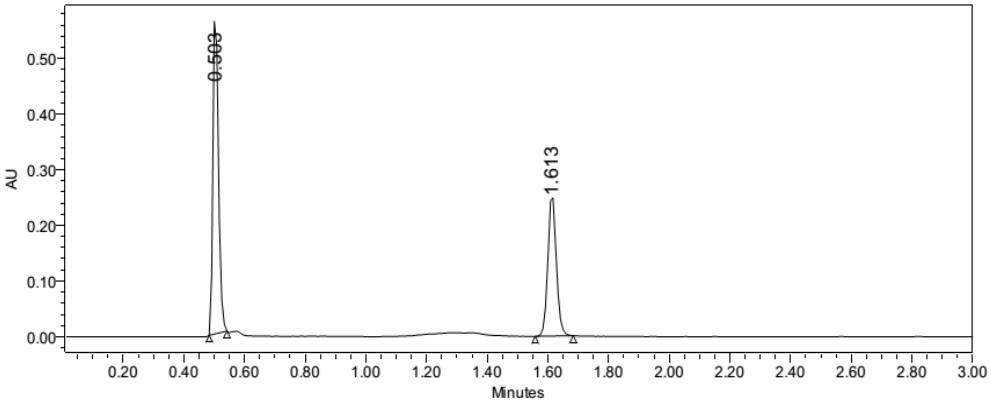


Fig. no.7- Chromatogram for the Intermediate Precision

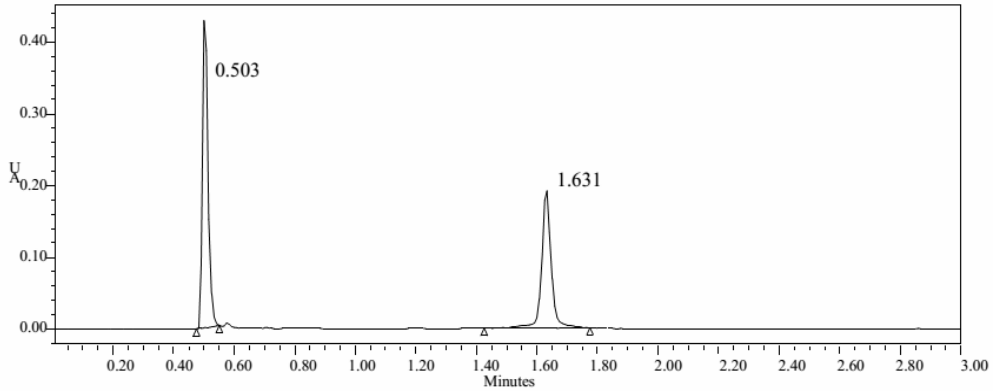


Fig. no.8- Chromatogram for the Accuracy (50%)

Table no.1: Precision result for the drug 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 no.2: Precision result for the drug 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

Table no.3: Ruggedness result for the drug Sitagliptin

Injection	**Area**
Injection-1	727720
Injection-2	713675
Injection-3	735699
Injection-4	727235
Injection-5	746709
Average	730207.6
Standard Deviation	12153.22
%RSD	1.664351

Table no.4: Ruggedness result for the drug Simvastatin

Injection	**Area**
Injection-1	494755
Injection-2	496592
Injection-3	491012
Injection-4	500433
Injection-5	479020
Average	492362.4
Standard Deviation	8194.386
%RSD	1.6643

Table no.5: Accuracy result for the drug 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 no.6: Accuracy result for the drug 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%	

Table no.7: Linearity result for the drug 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

Table no.8: Linearity result for the drug 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

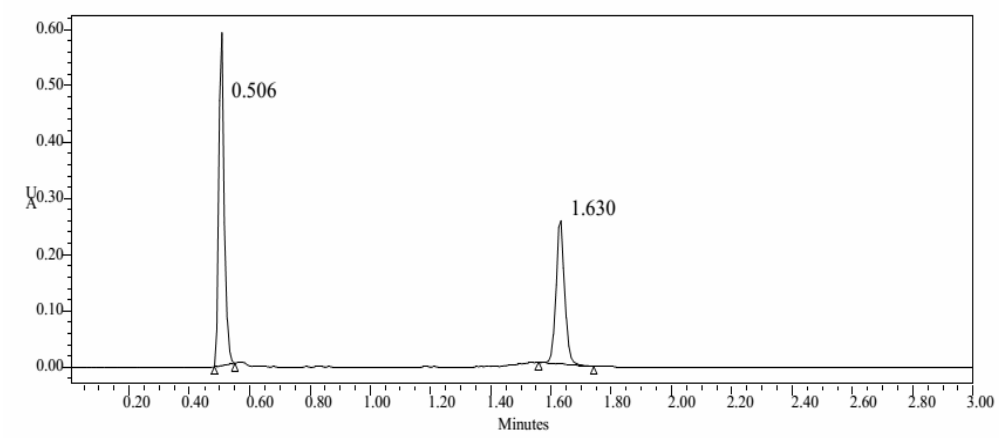


Fig. no.9- Chromatogram for the Accuracy (100%)

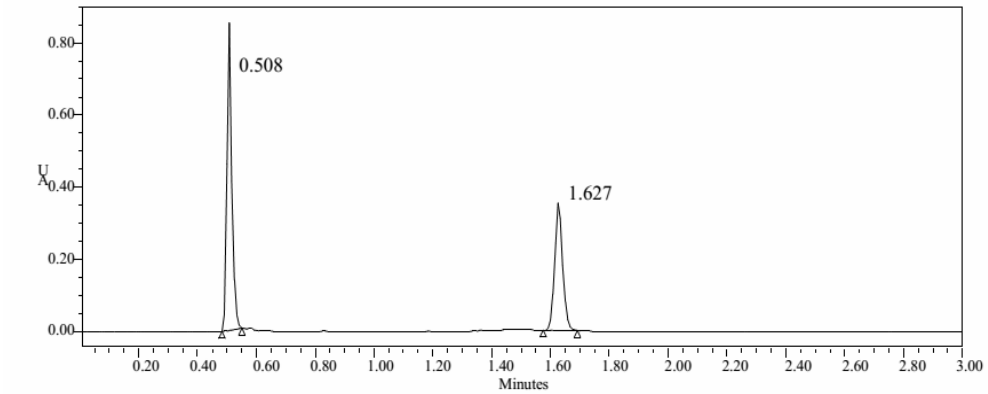


Fig. no.10 -Chromatogram for the Accuracy (150%)

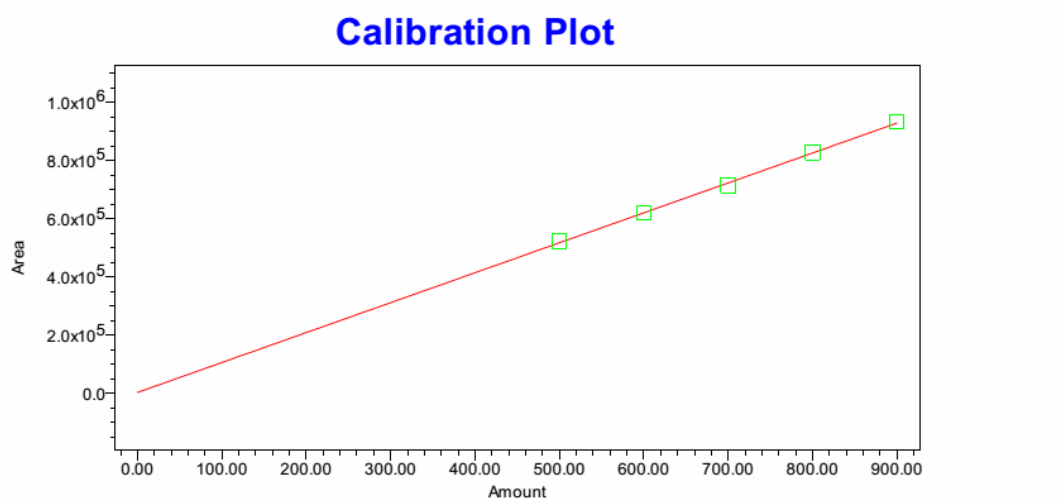


Fig. no. 11-Linearity curve for the drug Sitagliptin

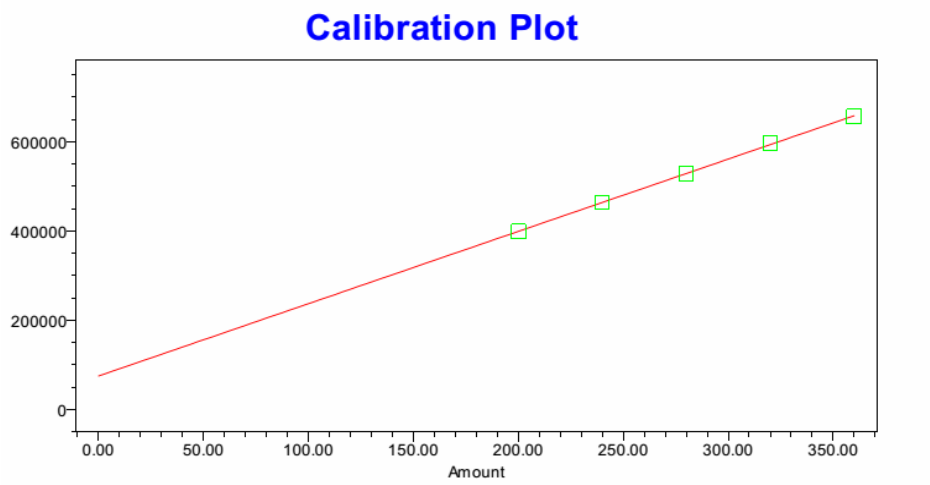


Fig. no. 12-Linearity curve for the drug Simvastatin

Results & Discussion

The present work was undertaken with the aim to develop and validate a rapid and consistent UPLC method development in which the peaks will be appear with short period of time as per ICH Guidelines. The proposed method was simple, fast, accurate and precise method for the Quantification of drug in the Pharmaceutical dosage form, bulk drug as well as for routine analysis in Quality control. Overall the proposed method was found to be suitable and accurate for the Quantitative determination of the drug in tablet dosage form. The method was simple, precise, accurate and sensitive and applicable for the simultaneous determination of Sitagliptin & Simvastatin in bulk drug and in combined dosage forms. The Ultra performance liquid chromatography (UPLC) methods was developed and validated for simultaneous estimation of Sitagliptin & Simvastatin in bulk drug and in combined dosage forms. The UPLC separation was achieved on a Symmetry C_{18} (2.1 x 100mm, 1.7µm, Make: BEH) or equivalent in an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (30%) whose pH was adjusted to 4.0 by using TEA & Acetonitrile (70%) [UPLC Grade]. The flow rate was monitored at 0.4 ml per min. The wavelength was selected for the detection was 213 nm. The run time was 3min. The retention time found for the drugs Sitagliptin & Simvastatin were 0.509 min. & 1.623 min. respectively. It was represented in fig. no. 4. The Precision data for the drugs Dutasteride & Tamsulosin were represented in Table no. 1 & 2 and the chromatograph was represented in Fig. No. 6. The %RSD for sample should be NMT 2. The %RSD for the standard solution was found to be 0.390062 & 0.954836 for the drugs Sitagliptin & Simvastatin respectively, which is within the limits hence the method was precise. When the drugs Sitagliptin & Simvastatin were analyzed by the proposed method in the intra and inter-day (Ruggedness) variation, a low coefficient of variation was observed it was represented in Table no. 3 & 4 and the chromatogram was represented in Fig. no.7 which shows that the developed RP-HPLC method was highly precise. The %RSD was found to be 1.664351 & 1.6643 for the drugs Sitagliptin & Simvastatin respectively, which is within the limits. The standard solution with Accuracy -50%, Accuracy -100% and Accuracy -150% were injected into chromatographic system and calculated the amount found and amount added for Sitagliptin &

Simvastatin and further calculated the individual recovery and mean recovery values. (Table no. 5 & 6). The chromatograms were represented in fig. no. 8, 9 &10. The % recovery was found to be 99.3%- 100.6% for the drug Sitagliptin. The % recovery was found to be 99.7% - 100.1% for the drug Simvastatin. In order to test the linearity of the method, five dilutions of the working standard solutions for the drugs Sitagliptin & Simvastatin were prepared. The linearity was established in the range of 500 to 900ppm for the drug Sitagliptin & 200 to360ppm for the drug Simvastatin. The data were represented in Table no. 7 & 8. Each of the dilution was injected into the column and the Linearity Curve was represented in Fig. no.11 & 12. The Correlation coefficient (R^2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which was in the acceptance limit. The Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The chromatograms were represented in Fig. no. 13 & 14. The LOD for the drugs Sitagliptin & Simvastatin were found to be 0.18µg/ml & 0.17µg/ml respectively. The LOQ for the drugs Sitagliptin & Simvastatin were found to be 0.61µg/ml & 0.56µg/ml respectively. The Signal to noise ratio should be 3 for LOD. The results obtained were within the limit. The Signal to noise ratio should be 10 for LOQ solution**.** The results obtained were within the limit. The Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in Phosphate Buffer and Acetonitrile in the mobile phase. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The system suitability parameters were within the limits and shown in Table No. 9, 10, 11 & 12 and chromatograms were represented in Fig. no. 15, 16, 17 & 18.

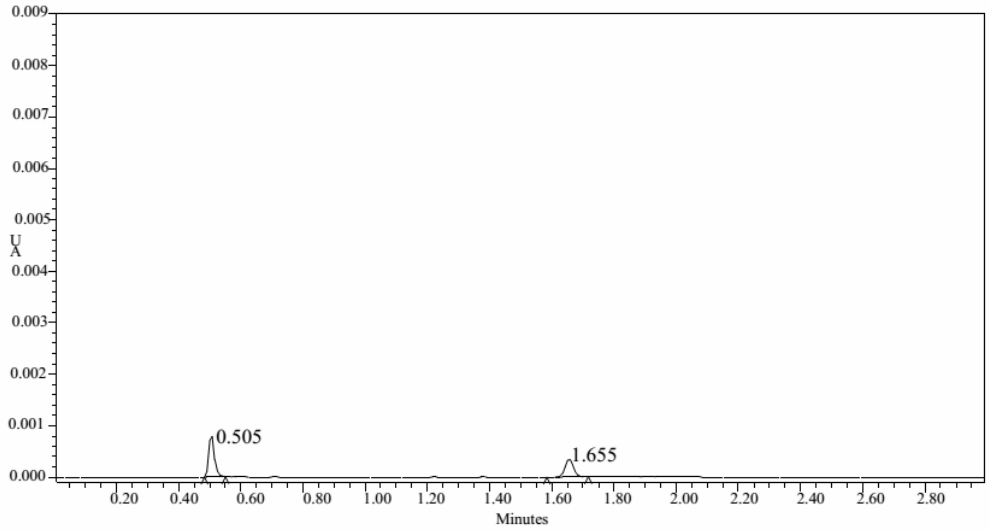


Fig. no. 13-Chromatogram for the drug Sitagliptin & Simvastatin (LOD)

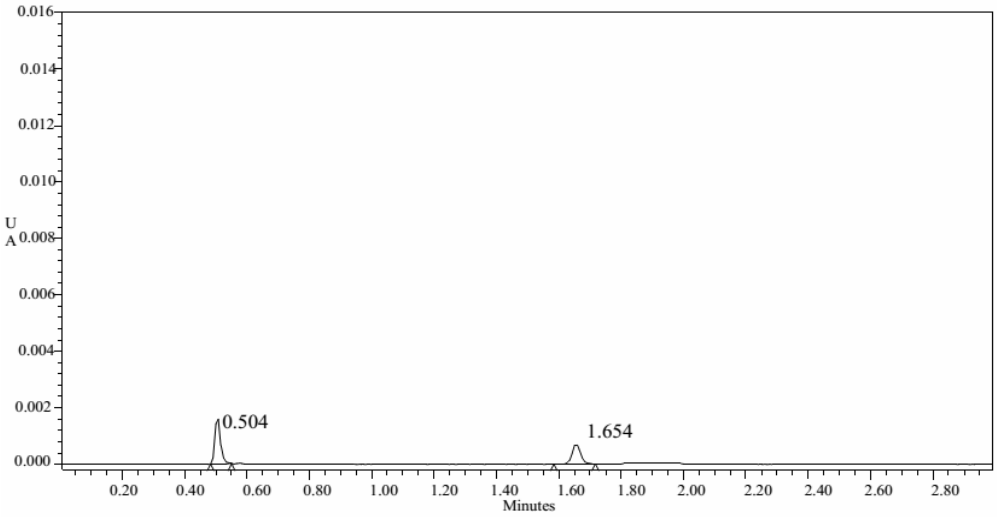


Fig. no. 14-Chromatogram for the drug Sitagliptin & Simvastatin (LOQ)

Table no.9: Result for effect of variation in flow rate for the drug Sitagliptin

Sr. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.3	3178.8	1.3
2	0.4	2556.4	1.3
3	0.5	2118.2	1.2

Table no. 10: Result for effect of variation in flow rate for the drug Simvastatin

Sr. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.3	15759.1	1.1
2	0.4	23185.6	1.1
3	0.5	11131.1	1.1

Table no.11: Result for effect of variation in mobile phase composition for the Drug Sitagliptin (Organic Phase)

Sr. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2487.3	1.2
2	Actual	2556.4	1.3
3	10% more	2341.5	1.1

Table no.12: Result for effect of variation in mobile phase composition for the Drug Simvastatin (Organic Phase)

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	16227.7	1.0
2	Actual	23185.6	1.1
3	10% more	11186.3	1.1

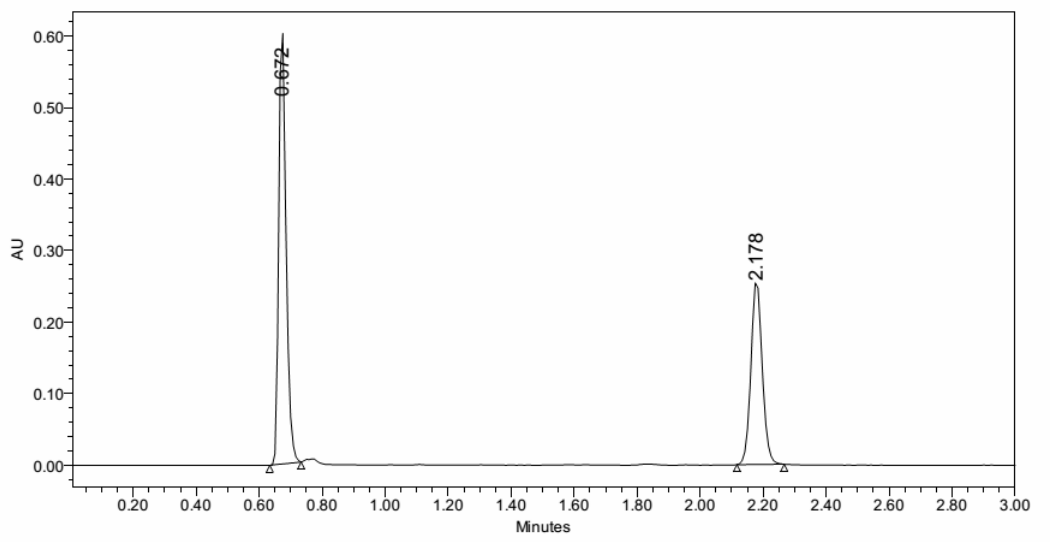


Fig. no. 15-Chromatogram for Less Flow Rate (0.3ml/min.)

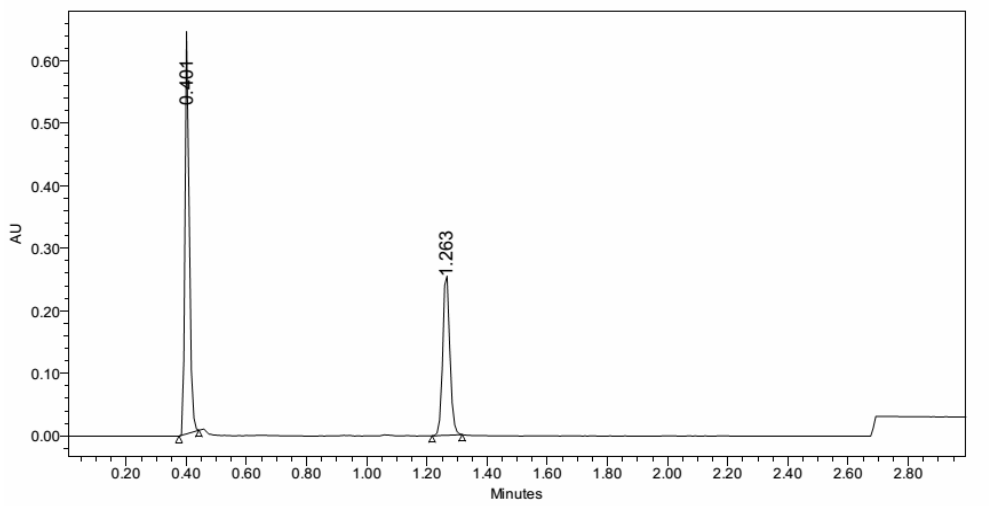


Fig. no 16- Chromatogram for More Flow Rate (0.5ml/min.)

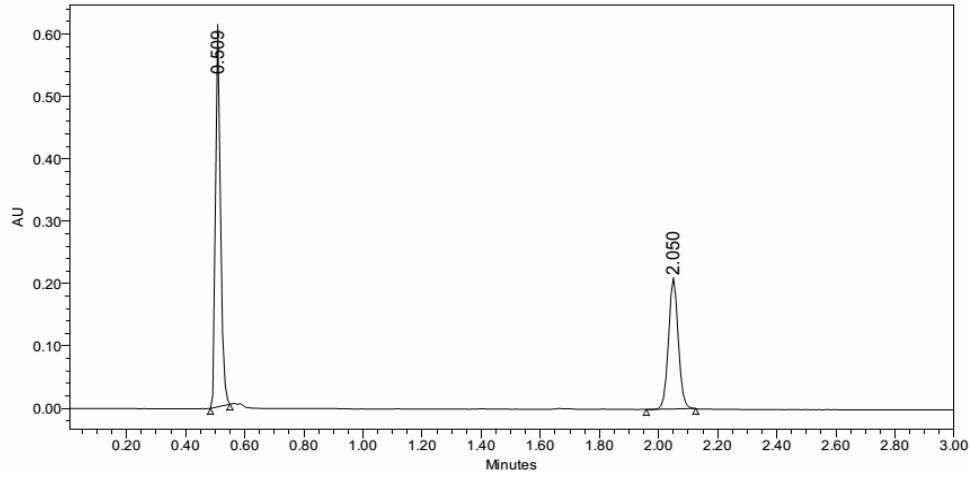


Fig. no. 17- Chromatogram for Less Organic Composition (60%)

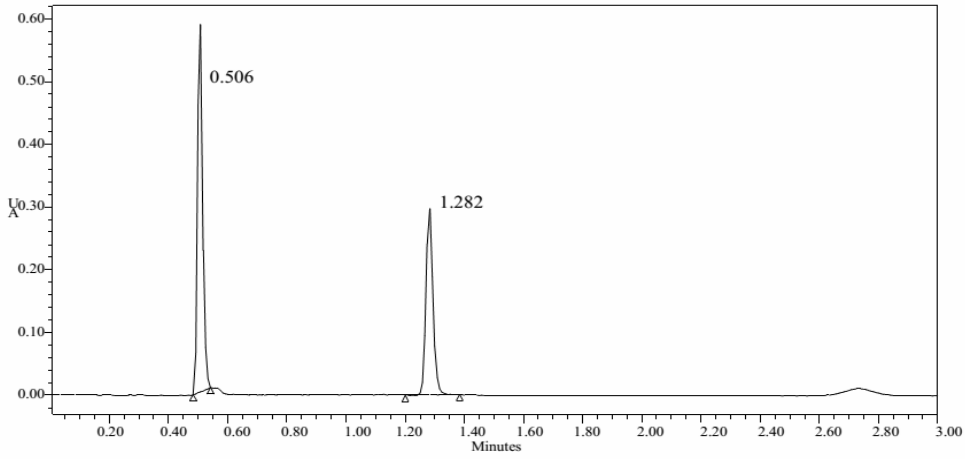


Fig. no. 18 -Chromatogram for More Organic Composition (80%)

Conclusion

Development of new analytical methods for the determination of drugs in pharmaceutical dosage is important in pharmacokinetic, toxicological biological studies. Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form.

The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs. It was concluded that the proposed new UPLC method developed for the quantitative determination of Sitagliptin & Simvastatin in bulk as well as in its formulations was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phases were simple to prepare and economical. The sample recoveries in the formulation 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 adopted as an alternative method to report routine determination of Sitagliptin & Simvastatin depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies for the drug Sitagliptin & Simvastatin. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases.

Future Aspect: The proposed method can be use in future for the clinical, biological and pharmacokinetic studies of Sitagliptin & Simvastatin.

References

1. Herman G, Bergman A, Liu F, Stevens C, Wang A, Zeng W et al.Pharmacokinetics and pharmacodynamic effects of the oral DPP-4 inhibitor Sitagliptin in middle-aged obese subjects.J Clin Pharmacol 2006; 46 (8): 876–886.
2. Dubal A, Khatwal R, Kosaraju J, Meda V, Samanta M. Bioanalytical method development and validation of Sitagliptin phosphate by RP-HPLC and its application to pharmacokinetic study. Int J Pharm Pharm Sci 2012; 4, (2): 691-694.
3. Balasekaran C, Rani P A. Development and validation of Spectrophotometric method for the determination of DPP-4 Inhibitor, Sitagliptin in its pharmaceutical preparations. Int J Pharm Pharm Sci 2010; 2(4): 138-142.
4. Parag P, Imran M, Bairagi V, Ahire Y. Development and validation of stability indicating UV Spectrophotometric method for the estimation of Sitagliptin phosphate in bulk and tablet dosage form. J Pharm Res 2011; 4(3): 871-873.
5. Khan, Agrawal Y P, Sabarwal N, Jain A, Gupta A K. Simultaneous Estimation of Metformin and Sitagliptin In tablet dosage form. Asian J Biochem Pharma Res 2011; 1(2): 352-358.
6. Ramzia E -Bagary, Ehab E F, Bassam A M. Spectroflourometric and Spectrophotometric methods for the determination of Sitagliptin in binary mixture with Metformin and ternary mixture with Metformin and Sitagliptin Alkaline Degradation Product. Int J Biomed Sci 2011; 7(1): 62-69.
7. Ravi P P, Sastry B S, Rajendra P Y, Appala R N. Simultaneous Estimation of Metformin HCl and Sitagliptin Phosphate in tablet dosage forms by RP-HPLC. Res J Pharm Tech 2011; 4(4): 646-649.
8. Shyamala M, Mohideen S, Satyanarayana T, Narasimha R, Suresh K, Swetha K. Validated RP-HPLC for simultaneous estimation of Sitagliptin phosphate and Metform in hydrochloride in tablet dosage form. American J Pharm Tech Res 2011; 1(2): 93-101.
9. Zeng W, Xu Y, Constanzer M, Woolf E J. Determination of Sitagliptin in human plasma using protein precipitation and tandem mass spectrometry. J Chromatogr B 2010; 878(21):1817-1823.
10. Zeng W, Musson DG, Fisher AL, Chen L, Schwartz MS, Woolf E J et al. Determination of Sitagliptin in human urine and hemodialysate using turbulent flow online extraction and tandem mass spectrometry. J Pharm Biomed Anal 2008; 46(3): 534-542.
11. Nirogi R, Kandikere V, Mudigonda K, Komarneni P, Aleti R, Boggavarapu R. Sensitive liquid chromatography tandem mass spectrometry method for the quantification of Sitagliptin, a DPP-4 inhibitor, in human plasma using liquid–liquid extraction. Biomed Chromatogr 2008; 22(2): 214–222.
12. Sharma Sheetal, Manocha Nimita, Bhandari Priya, Harsoliya Sohail and Jain Prabhat.Development of UV-Spectrophotometry and RP-HPLC Method and Its Validation for Simultaneous Estimation of Sitagliptin Phosphate and Simvastatin in Marketed Formulation. IJPBA 2012; 3(3): 673-678.
13. Kothari Ankur and Sheetal. Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin Phosphate and Simvastatin in Tablet dosage Form.JPR 2012; 5(1):444-446.
14. Phaneemdra D, Venkatesh, Ramarao N. Simultaneous estimation of Simvastatin and Sitagliptin by using different analytical methods. IJAPA 2012; 2(1):19-23.

15. Lough W J and Wainer I W W. HPLC Fundamental Principles and Practices, Blackie Academic and Professional; 1991. 52-67.
16. Gatti R, Lotti C. Development and validation of a pre-column reversed phase liquid chromatographic method with fluorescence detection for the determination of primary phenethylamines in dietary supplements and phytoextracts, Journal of Chromatography A, 2011; 1218(28):4468-4473. http://dx.doi.org/10.1016/j.chroma.2011.05.044. PMid: 21652038.
17. Ashutosh Kar. Pharmaceutical Drug Analysis, 2nded. New Age International Publishers; 2005. 466-467.
18. Sethi P D.HPLC Quantitative Analysis of Drug in Pharmaceutical Formulations; CBS publishers and distributors, 3rd Ed; 2001.101-103.
19. Dixit R P, Barhate C R, Padhye SG, Viswanathan C L, M S Nagarsenker. Stability indicating RP-HPLC method for simultaneous estimation of Simvastatin and Ezetimibe from tablet dosage form, IJPS2010; 72(2): 204-210.
20. Yaddanapudi Mrudula Devi, R. Karthikeyan, Punttaguntla Sreenivasa Babu. Analytical method development and validation for simultaneous estimation of Simvastatin and Sitagliptin. Int. Res. J. Pharm. 2013; 4(8):184-188. http://dx.doi.org/10.7897/2230-8407.04836.
21. S. Ashutosh Kumar, Manidipa Debnath, Dr. J.V.L.N.Seshagiri Rao. Simultaneous Estimation of Sitagliptin Phosphate Monohydrate and Metformin Hydrochloride in Bulk and Pharmaceutical Formulation by RP-HPLC. Am. J. Pharm Tech Res. 2013; 3(3), 556-575. Research article. ISSN: 2249-3387.
22. S. Ashutosh Kumar, Manidipa Debnath, Dr. J.V.L.N. Seshagiri Rao. Development of Stability Indicating RP- HPLC method for Simultaneous Estimation of Metformin Hydrochloride and Sitagliptin Phosphate Monohydrate in Bulk as well as in Pharmaceutical formulation. Der Pharmacia Sinica, 2013, 4(4):47-61. ISSN: 0976-8688.
23. Indian Pharmacopeia, Volume I, Published by The Indian Pharmacopoeia Commission, 477-478; 2007.
24. Yuri Kazakevich and Rosario Lobrutto Seton. HPLC for Pharmaceutical Scientists. 1st Ed. Published by Wiley-VCH; 2007; 369-382.
25. Stavros Kromidas. HPLC Made to Measure, A Practical Book for Optimization. Published by Wiley-VCH; 2006; 62-66.
26. Yuri Kazakevich and Rosario Lobrutto Seton. HPLC for Pharmaceutical Scientists. 1st Ed. Published by Wiley-VCH; 2007; 389-391.
27. Jenke D.R. Chromatographic method validation: A review of current practices and Procedures. II. Guidelines for primary validation parameters. J. Liq. Chromatogr.19, 737–757, 1996.
28. Sharma Ajay, Sharma Rohit: Validation of analytical procedures: a comparison of ICH VS PHARMACOPOIEA (USP) and FDA. Inter National Research Journal of Pharmacy 2012; 3(6) 39-42.
29. Validation of analytical procedure: Methodology Q2B, ICH Harmonized Tripartite Guidelines, 1996:1-8.
30. International Conference on Harmonization of Technical Requirements for Registration ofPharmaceuticals for Human Use ICH Harmonized tripartite guideline Validation of analytical procedures: Text and Methodology Q2 (R1) 6 November 1996.
31. Ravichandran V, Shalini S, Sundram K. M and Harish Rajak: Validation of analytical methods – strategies& importance International Journal of Pharmacy and Pharmaceutical Sciences 2010; vol 2, suppl 3,
32. Tangri Pranshu, Rawat Prakash Singh, Jakhmola Vikash: Validation: A Critical Parameter for Quality Control of Pharmaceuticals. Journal of Drug Delivery & Therapeutics 2012; 2(3): 34- 40.
33. ICH harmonized tripartite guideline. Impurities in New Drug products Q3B (R2) current step 4 versions dated 2 June 2006.
34. ICH Harmonizes Tripartite Guideline, Validation of analytical procedures: Text and Methodology Q2 (R1), November, 2005.

*****