International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.14 | Issue 3 | Jul - Sept -2025

www.ijpar.com

ISSN: 2320-2831

DOI : https://doi.org/10.61096/ijpar.v14.iss3.2025.577-593

Research

Development of New Simple, Sensitive, Accurate and Economical Analytical for the Quantitative Determination of Sitagliptin and Simvastatin in Pure Form and its Pharmaceutical Dosage Form

K. Srivani*, M. Bhaskar [1], N. Sanhya Rani[1], G.Bhavai[1]

Smt. Sarojini Ramulamma College of Pharmacy, Seshadri Nagar, Mahabub Nagar-509001, Telangana, India.

*Author for Correspondence: K. Srivani
Email: srivanikarne10@gmail.com

Published on: 09 Sep 2025

Published by:
Futuristic Publications

Abstract

Sitagliptin is an oral anti-hyperglycemic agent of the dipeptidyl peptidase-4 (DPP-4) inhibitor class used in the treatment of type-2 diabetes. It stimulates insulin release and reduces glucagon level by inhibition of inactivation of the incretin a glucose-dependant manner. In 2013 it was the second best-selling drug in the U.S. This review explores the reported analytical methods so far in the literature for the estimation of Sitagliptin as we as Simvastatin in bulk drug, pharmaceutical formulations and in biological matrix. This assessment encompasses various analytical methods such as spectrometry, high performance liquid chromatography (HPLC), liquid chromatography for the estimation of Sitagliptin and Simvastatin in single and/or in combination.

Keywords: Sitagliptin, Simvastatin, Analytical methods, Type-2 diabetes.

INTRODUCTION

The study of composition, structure and properties of atoms or molecules is known as chemistry. Chemistry is the branch of physical science that includes Organic chemistry, Inorganic chemistry and Analytical chemistry. Organic chemistry deals with synthesis of drugs.[1]The instrumental analysis involves the spectroscopic and chromatographic techniques.

Spectroscopy
Atoms when they emit radiation there produce a spectrum which is measured by spectroscopy.
Chromatography
The phenomenon of separation of mixture of compounds is known as chromatography.
Spectroscopic Techniques
Ultraviolet Spectroscopy, Nuclear Magnetic Resonance Spectroscopy,Infrared Spectroscopy and Mass Spectroscopy.

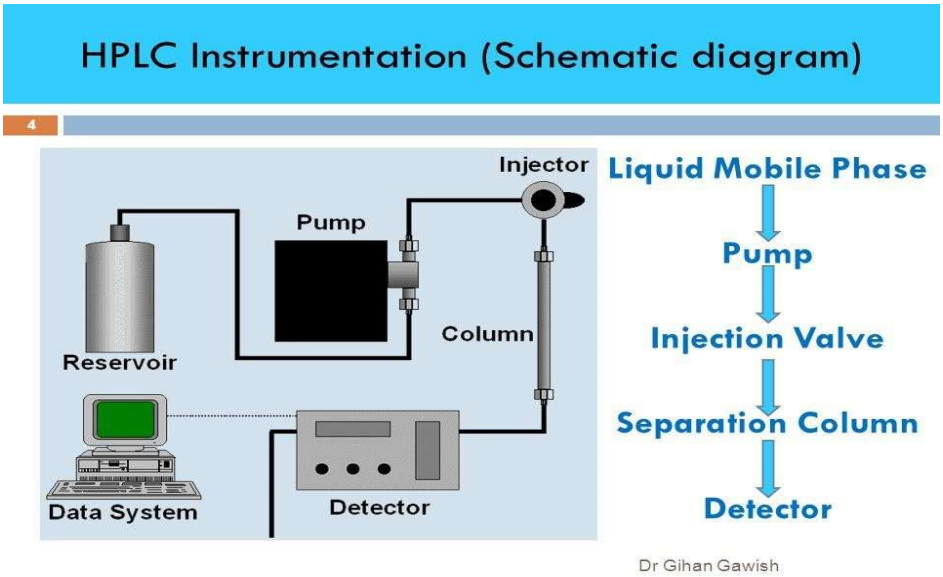
Chromatographic Techniques
Gas chromatography, Thin layer chromatography, High performance thin layer chromatography, Paper chromatography and High performance liquid chromatography.[2]

Instrumentation
Light source: The mostly used source of light should be stable and ntense. Mostly used sources of light are Xenon lamp, Hydrogen lamp and Deuterium lamp.
Monochromator: Monochromators are used to convert polychromatic light In to monochromatic light.
Sample cells: Sample cells are of quartz or glass, they are used to hold the samples.
Detector: They are used to detect the sample absorbance detectors used are
Photo voltaic cell and Photo emissive cell and Photo multiplier tube.[3]

Detection of functional groups
The presence or absence of chromophore is detected by UV Spectrophotometer. Chromophore in the complex compounds is not detected by UV Spectrophotometer.
Extent of conjugation
In polymers extent of conjugation is detected by UV spectrophotometer.
Detection of unknown compounds
The spectrums of unknown compounds are compared with the reference compound spectrum and it confirms the unknown substance.
Used to detect the absorbance of the sample. It is used to detect the wave length of the sample (λ max).

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 is highly performed by diffusion process. It has high speed, efficient, high resolution for separation of mixture of compounds, when compared to other techniques.

Basic Instrumentation



HPLC Types of HPLC based

Based on mobile phases and stationary phases
1. Reverse phase HPLC
2. Normal phase HPLC

Reverse phase HPLC
In reverse phase HPLC, we use polar mobile phase and non-polar stationary phase. Mostly we use reverse phase

HPLC for the separation and analyzing the purity.

Normal phase HPLC

In normal phase HPLC we use polar stationary phase and a non-polar mobile phase.

Types of HPLC based on modes of separation 1.Isocratic mode and 2.Gradient mode

Isocratic mode of HPLC

In throughout the process of Separation the composition of mobile phase using will be constant. In this mode single pump analysis can be done.

Gradient mode of HPLC

In throughout the process of separation the composition of mobile phase .In this mode multiple pump analysis can be done.

Components of the HPLC [4]

- Reservoir
- Pump loops
- Draining valve
- Sample holding vials
- Auto samplers
- Guard column
- Column
- Detector
- Recorder

Separation parameters in hplc include

- Column length
- Mobile phases
- Stationary phase
- Organic compound present in mobile phase
- Temperature of the column
- Flow rate of the mobile phase

Analytical Method Development [5]

Analytical method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantify and purify compounds. Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use". Understanding the physical and chemical characteristics of a drug allows, to select the most appropriate high performance liquid chromatography method from the available vast literature. Information concerning the sample, for example, molecular mass, structure and functionality, pKa values, UV spectra, solubility of compound should be compiled. The requirement of removal of insoluble impurities by filtration, centrifugation, dilution derivatization for detection etc. should be verified. For pure compound, the sample solubility should be identified as this helps to select the mobile phase and column to run an new HPLC method. Method development in HPLC can be laborious and time consuming to optimize a method on a column to accomplish the goals. Even among reversed phase columns, there is astonishing diversity, owing to differences in both silica and bonded phase characteristics. The three critical components for a HPLC method are: sample preparation (% organic, pH, shaking / sonication, sample size, sample age) analysis conditions (pH, flow rate, temperature, wavelength, and column age), and standardization (integration, wavelength, standard concentration, and response factor correction). During the preliminary method development stage, all individual components should be investigated before the final method optimization.

Validation Method [6-9]

The validation of an analytical method demonstrates the scientific soundness of the measurement or characterization. It is required to provide validation data throughout the regulatory submission process. The validation practice demonstrates that an analytical method measures the correct substance, in the correct amount, and in the appropriate range for the intended samples. It allows the analyst to understand the behavior of the method and to establish the performance limits of the method. The goal is to identify the critical parameters and to establish acceptance criteria for method system suitability.

Validation is defined by the International Organization for Standardization (ISO) as "verification, where the specified requirements are adequate for an intended use", where the term verification is defined as "provision of objective evidence that a given item fulfills specified requirements". The applicability and scope of an analytical method should be defined before starting the validation process. It includes defining the analyte, concentration range, description of equipment and procedures, validation level and criteria required. The validation range is defined by IUPAC as "the interval of analyte concentration within which the method can be regarded as validated". This range is not the highest and lowest possible levels of the analyte that can be determined by the method. Instead, it is defined on the basis of the intended purpose of the method. It can also be validated for use on single equipment, different equipments in the laboratory, different laboratories or even for international use at different climatic and environmental conditions. The criteria of each type of validation will of course be different with the validation level required. The various validation parameters include linearity, accuracy, precision, ruggedness, robustness, LOD, LOQ and selectivity or specificity.

Aim

In view of the need for a suitable RP-HPLC method for routine analysis of Sitagliptin and Simvastatin in combined formulations, various trials were performed to develop simple, precise and accurate method for quantitative determination of Sitagliptin and Simvastatin..

Validation is a necessary and important step in both framing and documenting the capabilities of the developed method.

The utility of the developed method to determine the content of Sitagliptin and Simvastatin in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient. The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, limit of detection and limit of quantification. The resultant criteria upon validating, proposes the method as suitable for the analysis of Pharmaceutical dosage forms.

Objective

To develop new simple, sensitive, accurate and economical analytical for the quantitative determination of Sitagliptin and Simvastatin in pure form and its pharmaceutical dosage form.

To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Sitagliptin and Simvastatin in pharmaceutical dosage form.

Methodology

Table 1: List Chemicals used

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

Method Development [10-11]

Trails

Preparation of standard solution

10 mg of Sitagliptin and Simvastatin pure drugs was weighed and transferred into a 10ml of clean dry volumetric flasks, 7ml of diluent was added to each and sonicated to dissolve and remove air completely and volume was made up to the mark with methanol. Further 0.1ml of the above Sitagliptin and 0.3ml of the Simvastatin stock solutions were pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure

The samples were injected by changing the chromatographic conditions, the chromatograms were recorded and the conditions of proper peak elution for performing validation as per ICH guidelines were noted**.**

Mobile Phase Optimization

Initially the mobile phase tried was CAN

Water and Water: Methanol and Methanol: TEA Buffer and ACN: Phosphate buffer with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Buffer in proportions of 45:55 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Phenomenex Luna C18 (4.6×250mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized Chromatographic Conditions

- Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
  Temperature : 35ºC
- Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
- Buffer : Dissolve 6.8043g of potassium dihydrogen phosphate in 1000 ml HPLC grade water and adjust the pH to 4.6 with diluted orthophosphoric acid. The buffer was filtered,
- pH : 4.6
- Mobile phase : Acetonitrile: Phosphate Buffer (45:55
  v/v) Flow rate : 1ml/min
- Wavelength : 245 nm Injection volume :
- 10 □l Run time : 7 min

Preparation of Buffer and Mobile Phase

Preparation of Potassium dihydrogen Phosphate (KH_2PO_4) buffer (pH-4.6)

6.8043g of potassium dihydrogen phosphate was dissolved in 1000ml HPLC grade water and the pH was adjusted to 4.6 with diluted orthophosphoric acid. The solution was filtered.

Preparation of mobile phase

450ml of ACN, 550ml of Phosphate buffer were measured and mixed together and the solution was degassed for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Assay [12,31]

Preparation of standard solution

10 mg of Sitagliptin and Simvastatin pure drugs was weighed and transferred into a 10ml of clean dry volumetric flasks, 7ml of diluent was added to each and sonicated to dissolve and remove air completely and volume was made up to the mark with methanol. Further 0.1ml of the above Sitagliptin and 0.3ml of the Simvastatin stock solutions were pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of Sample Solution

Average weight of 5 Tablets was taken and crushed in a mortar by using pestle and weigh 10 mg equivalent weight of Sitagliptin and Simvastatin sample into a 10ml clean dry volumetric flask and add about 7ml of diluents and sonicated to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 0.1ml of the above Sitagliptin and 0.3ml of the Simvastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

%ASSAY =

$$\frac{\text{Avg area of sample}}{\text{Avg area of sample}} \times \frac{\text{wt of std}}{\text{Dilution}} \times \frac{1}{\text{Dilution}} \times \frac{\text{wt of sample}}{\text{Dilution}} \times \frac{\text{Dilution}}{1} \times \frac{\text{Avg wt of Tablet}}{\text{Label claim}} \times 100$$

Validation Parameters [13-14]

System Suitability

Preparation of standard solution

10 mg of Sitagliptin and Simvastatin pure drugs was weighed and transferred into a 10ml of clean dry volumetric flasks, 7ml of diluent was added to each and sonicated to dissolve and remove air completely and volume was made up to the mark with methanol. Further 0.1ml of the above Sitagliptin and 0.3ml of the Simvastatin stock solutions were pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure
The standard solution was injected for five times and the peak area was measured for all five injections. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity [17,30]
Preparation of Standard Solution
10 mg of Sitagliptin and Simvastatin pure drugs was weighed and transferred into a 10ml of clean dry volumetric flasks, 7ml of diluent was added to each and sonicated to dissolve and remove air completely and volume was made up to the mark with methanol. Further 0.1ml of the above Sitagliptin and 0.3ml of the Simvastatin stock solutions were pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of Sample Solution
Average weight of 5 Tablets was taken and crushed in a mortar by using pestle and weigh 10 mg equivalent weight of Sitagliptin and Simvastatin sample into a 10ml clean dry volumetric flask and add about 7ml of diluents and sonicated to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 0.1ml of the above Sitagliptin and 0.3ml of the Simvastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Linearity[15-16]
Preparation of Stock Solution
10 mg of Sitagliptin and Simvastatin pure drugs was weighed and transferred into a 10ml of clean dry volumetric flasks, 7ml of diluent was added to each and sonicated to dissolve and remove air completely and volume was made up to the mark with methanol. Further 0.1ml of the above Sitagliptin and 0.3ml of the Simvastatin stock solutions were pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 6µg/ml of Sitagliptin & 18µg/ml of Simvastatin solution:
0.06ml of Sitagliptin and 0.18ml of Simvastatin was pipetted out from the stock solutions into a 10ml volumetric flask and diluted up to the mark.

Preparation of 8µg/ml of Sitagliptin & 24µg/ml of Simvastatin solution:
0.08ml of Sitagliptin and 0.24ml of Simvastatin was pipetted out from the stock solutions into a 10ml volumetric flask and diluted up to the mark.

Preparation of 10µg/ml of Sitagliptin & 30µg/ml of Simvastatin solution:
0.1ml of Sitagliptin and 0.3ml of Simvastatin was pipetted out from the stock solutions into a 10ml volumetric flask and diluted up to the mark.

Preparation of 12µg/ml of Sitagliptin & 36µg/ml of Simvastatin solution:
0.12ml of Sitagliptin and 0.36ml of Simvastatin was pipetted out from the stock solutions into a 10ml volumetric flask and diluted up to the mark.

Preparation of 14g/ml of Sitagliptin & 42µg/ml of Simvastatin solution:
0.14ml of Sitagliptin and 0.42ml of Simvastatin was pipetted out from the stock solutions into a 10ml volumetric flask and diluted up to the mark.

Procedure
Inject each concentration range solutions into the chromatographic system and run the chromatogram to measure the peak area.
Plot a graph between concentration and peak area on X-axis and Y-axis respectively, and calculate the slope and correlation coefficient.

Precision Repeatability [18 -5]
Preparation of Sitagliptin and Simvastatin Standard Solution for Precision
10 mg of Sitagliptin and Simvastatin pure drugs was weighed and transferred into a 10ml of clean dry volumetric flasks, 7ml of diluent was added to each and sonicated to dissolve and remove air completely and volume was made up to the mark with methanol. Further 0.1ml of the above Sitagliptin and 0.3ml of the Simvastatin stock solutions were pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent.

The standard solution was injected for five times and peak areas were measured.
The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Day 1: The standard solution was injected for six times and the area was measured for six injections. The %RSD for the area of six replicate injections was found to be within the specified limits.
Day 2: The standard solution was injected for six times and the area was measured for six injections. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy [26-29]

For preparation of 50% 100% 150% Standard stock solution

10 mg of Sitagliptin and Simvastatin pure drugs was weighed and transferred into a 10ml of clean dry volumetric flasks, 7ml of diluent was added to each and sonicated to dissolve and remove air completely and volume was made up to the mark with methanol. Further 0.1ml of the above Sitagliptin and 0.3ml of the Simvastatin stock solutions were pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent.

RESULTS AND DISCUSSION

Trail 1

Mobile phase : ACN: Water (80:20%v/v)
Column : Symmetry C18 (4.6 ×150mm, 5µm particle size) Make; waters
Flow rate : 0.8ml/min
Wavelength : 245 nm
Column temp :40ºC
InjectionVolume:10µl
Run time : 4 minutes

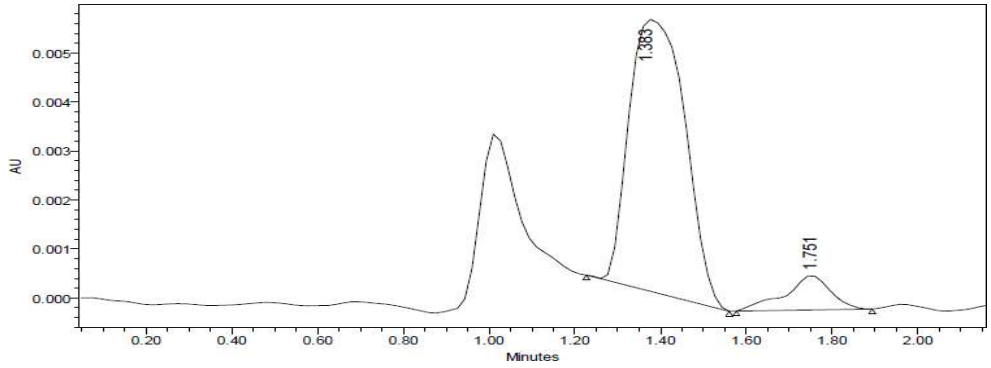


Fig 1: Chromatogram for Trail 1

Table 2: Peak results for Trail 1

S.No	Peak Name	Rt	Area	Height	USP Resolution	USP Tailing	USP Plate count
1	Sitagliptin	2.258	1256365	753652	------	0.86	785

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 trials2.

Trail 2

Mobile phase : Water and
Methanol (40:60v/v)

Column : Zodiac C18
(4.6×250mm) 5µ
Flow rate : 0.8 ml/min
Wavelength : 245 nm
Column temp : 40ºC
Injection Volume : 8 µl
Run time : 2 minutes

Table 3: Peak results for trail 2

S. No	Peak name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Sitagliptin	1.383	5632548	5689		1.23	658
2	Simvastatin	1.751	6958	878	1.9	0.83	895

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.

Trail 3:
Mobile phase : Methanol: TEA Buffer pH 3.2
(40:60% v/v) Column : Zodiac C18 (4.6×250mm 5µm)
Flow rate : 1.0 ml/min
Wavelength : 245 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 10 minutes

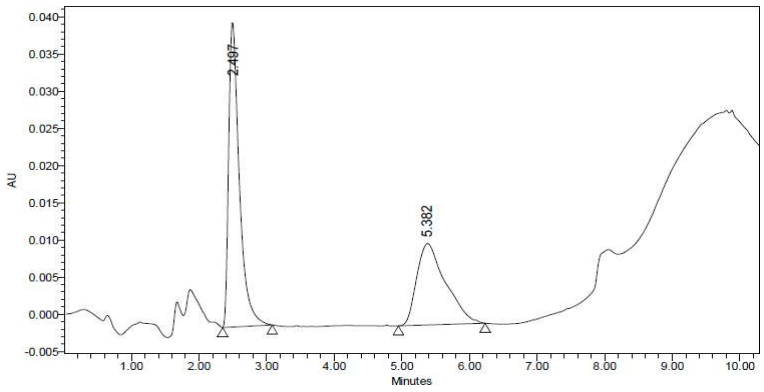


Fig 2 Chromatogram for trail 3

Table 4: peak results for trail 3

S. No	Peak name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Sitagliptin	2.497	456582	40865		2.5	1365
2	Simvastatin	5.382	33254	10784	1.39	3.5	698

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.

Trail 4:

Mobile phase : Acetonitrile: Phosphate Buffer (pH-3.6) (30:70 v/v) Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size Flow rate : 1 ml/min
Wavelength : 245 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 7 minutes

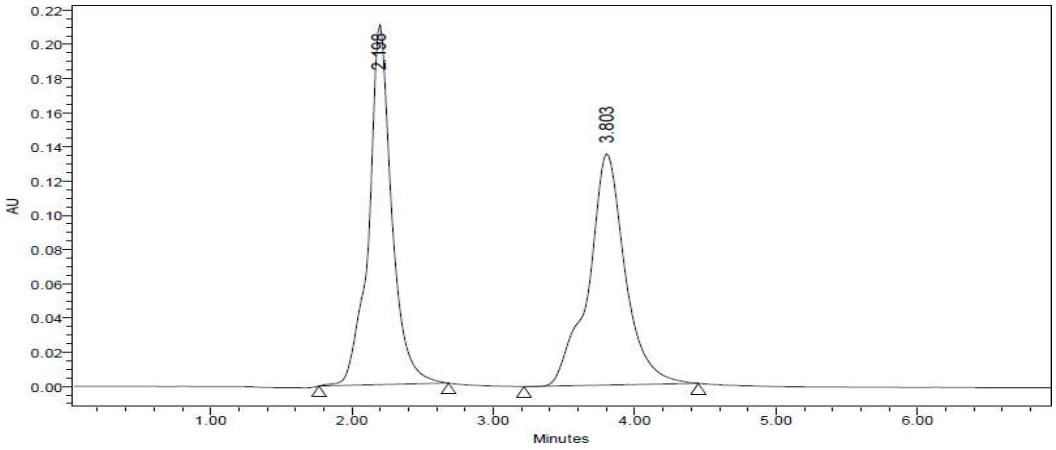


Fig 3: Chromatogram for trail 4

Table 5: Peak results for trail 4

S. No	Peak name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate Count
1	Sitagliptin	2.198	2458478	212541		1.0	1564
2	Simvastatin	3.803	2465814	136534	3.02	1.0	1452

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.

Trail 5

Mobile phase : Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v) Column : Phenomenex Luna C18 (4.6×250mm, 5µm)
particle size Flow rate : 1 ml/min
Wavelength : 245 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 7 minutes

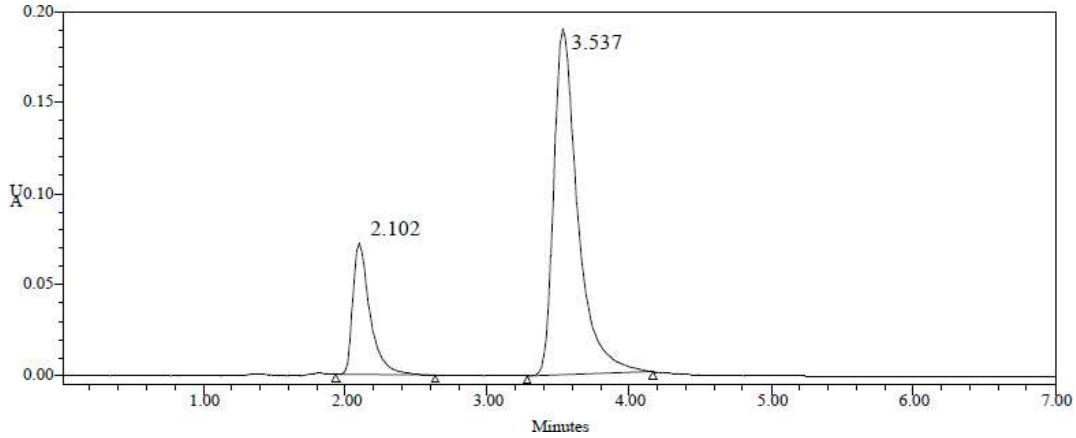


Fig 4: Chromatogram for Trail 5

Table 6: Peak results for Trail 5

S. No	Peak name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Sitagliptin	2.102	765789	69584		0.97	5587.0
2	Simvastatin	3.537	2532158	190049	2.97	1.26	5398.0

Observation: From the chromatogram we observe that the separation of two compounds was good with high resolution, base line was also good, retention time is also less and theoretical plate count is within the acceptance criteria. So we have gone for the repeatability of this method with same chromatographic conditions for optimization.

ASSAY (Standard)

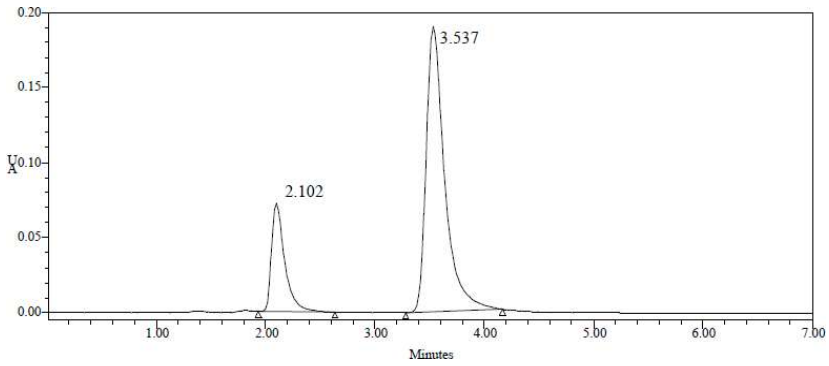


Fig 5: Chromatogram showing assay of standard injection -1

Table 7: Peak results for assay standard

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

Assay (Sample)

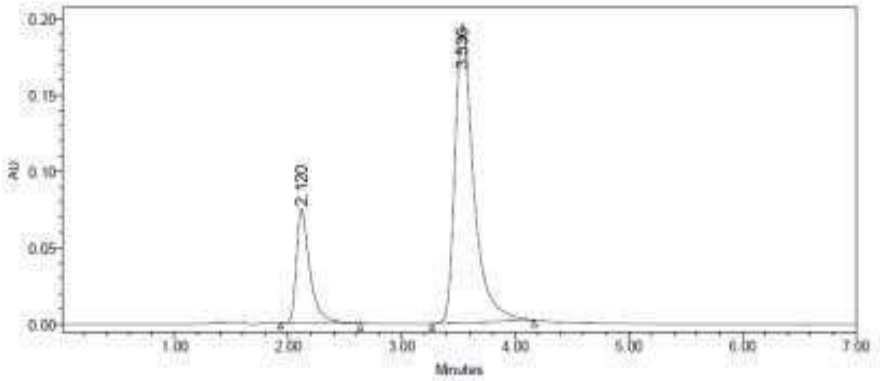


Fig 6: Chromatogram showing assay of sample injection-1

Table 8: Peak results for Assay sample

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

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Wt of Standard}}{\text{Dilution}} \times \frac{\text{Dilution}}{\text{Wt of Sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Wt of tablet}}{\text{Label claim}} \times 100$$

Observation: The % purity of Sitagliptin and Simvastatin in pharmaceutical dosage form was found to be 99.8%.

Validation
Blank

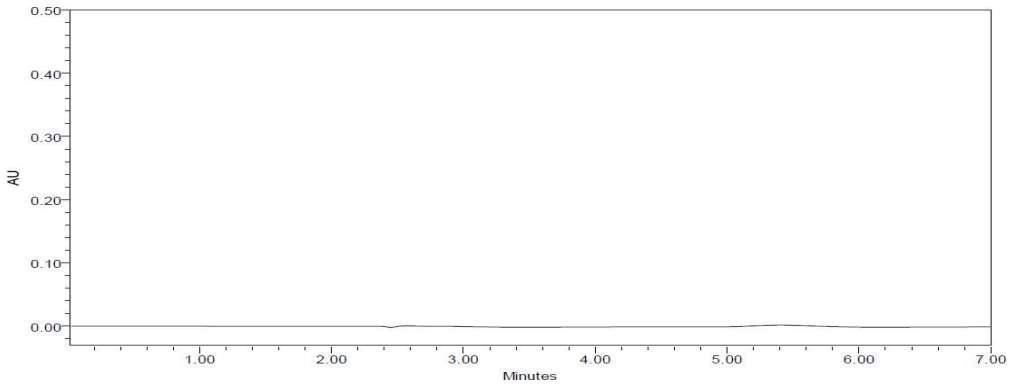


Fig 7: Chromatogram showing blank (mobile phase preparation)

System suitability

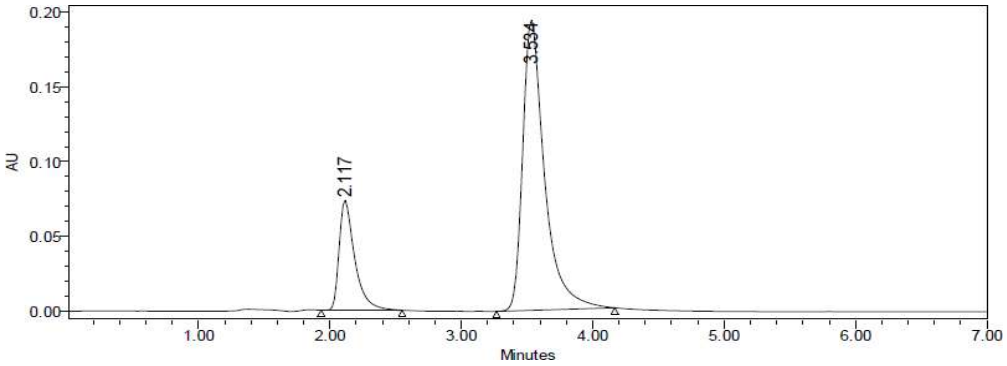


Fig 8: Chromatogram showing injection -1

Table 9: Results of system suitability for Sitagliptin

S.No	Name	Rt	Area	Height	USP plate Count	USP Tailing
1	Sitagliptin	2.117	765843	69587	5589	1.9
2	Sitagliptin	2.118	766594	69854	5576	1.6
3	Sitagliptin	2.116	765487	70211	5658	1.6
4	Sitagliptin	2.109	765928	69213	5642	1.7
5	Sitagliptin	2.102	765426	69558	5685	1.6
Mean			765855.6			
Std. Dev			466.6522			
% RSD			0.060932			

Acceptance criteria

%RSD for sample should be NMT 2.

The %RSD obtained is within the limit, hence the method is suitable.

Table 10: Results of system suitability for Simvastatin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Simvastatin	3.547	2534658	190058	5365	1.2	2.07
2	Simvastatin	3.539	2536854	190052	5348	1.4	2.05
3	Simvastatin	3.547	2535879	190078	5389	1.5	2.0
4	Simvastatin	3.565	2533564	190035	5347	1.6	2.01
5	Simvastatin	3.537	2534214	190085	5364	1.6	2.01
Mean			2535034				
Std. Dev			1183.309				
% RSD			0.046678				

Acceptance criteria

%RSD for sample should be NMT 2.

The %RSD obtained is within the limit, hence the method is suitable.

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitated Sitagliptin and Simvastatin in drug product.

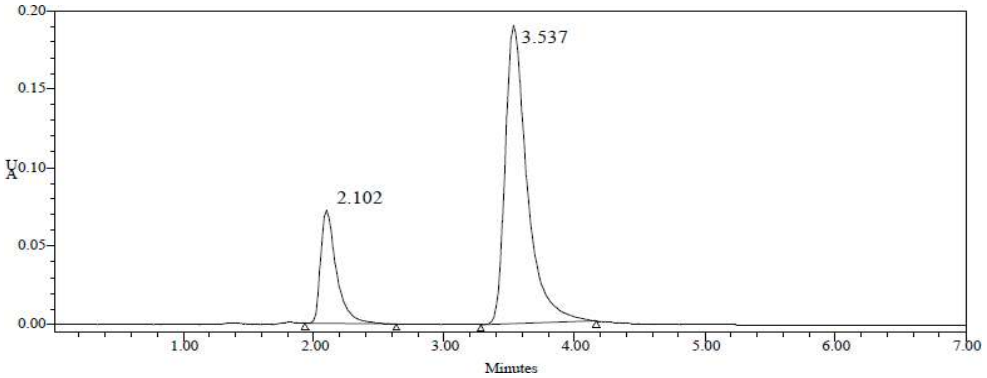


Fig 9: Chromatogram of standard injection -1

Table 11: Specificity results for standard

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

Sample

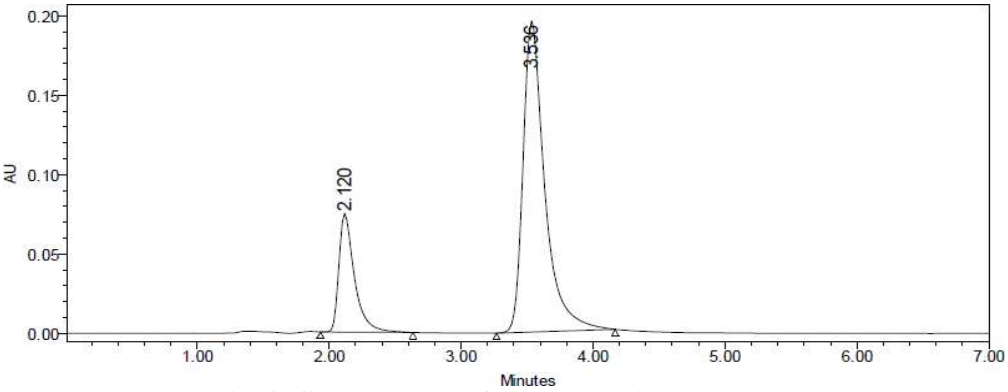


Fig 10: Chromatogram of sample injection-1

Table 12: Specificity results for sample

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

All the parameters of the chromatograms are within the limits for standard and sample of both the drugs. Hence from the result we can say our method is specific.

Linearity

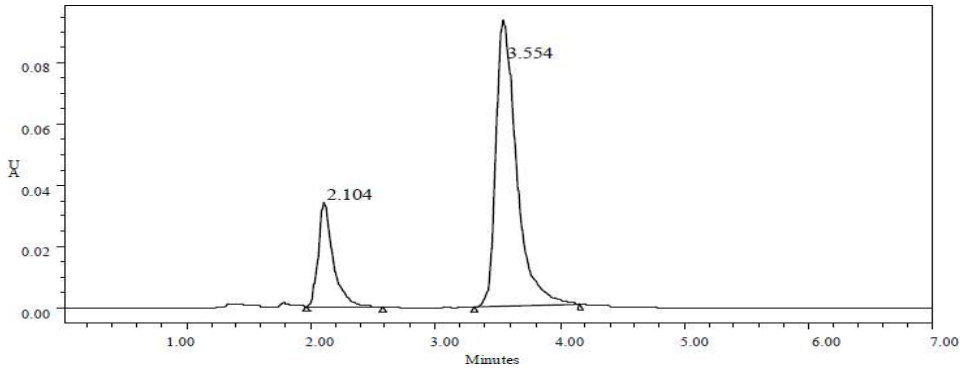


Fig 11: Chromatogram for 6µg/ml of Sitagliptin & 18 µg/ml of Simvastatin

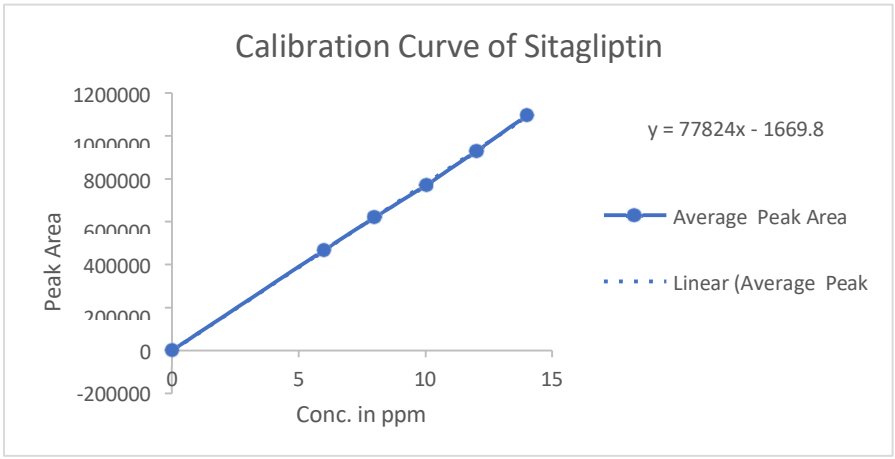


Fig 12: Calibration Graph for Sitagliptin

Linearity Plot
The plot of Concentration (x) versus the Average Peak Area (y) data of Sitagliptin is a straight line.
Y = mx + c
Slope (m) = 77824
Intercept (c) = 1669
Correlation Coefficient (r) = 0.999
Acceptance criteria: The response linearity is verified, if the Correlation Coefficient is 0.99 or greater.
Observation: Correlation Coefficient (r) is 0.99, and the intercept is 1669. These values meet the acceptance criteria.

Linearity Plot
The plot of Concentration (x) versus the Average Peak Area (y) data of Simvastatin is a straight line.
Y = mx + c
Slope (m) = 10515
Intercept (c) = 45591
Correlation Coefficient (r) = 0.999
Acceptance criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Observation: Correlation Coefficient (r) is 0.99, and the intercept is 45591. These values meet the validation criteria.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Acceptance criteria

%RSD for sample should be NMT 2

The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 13: Results of Repeatability for Simvastatin

S no	Name	Rt	Area	Height	USP plate count	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

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 14: The accuracy results for Sitagliptin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	204962	15	15.156	101.040%	100.93%
100%	365018	30	30.378	101.260%	
150%	521064.3	45	45.218	100.484%	

The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

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, flow rate was 1ml/min, mobile phase ratio was (45:55 v/v) Acetonitrile: Phosphate Buffer (pH-4.6 was adjusted with orthophosphoric acid), detection wave length was 245nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.102mins and 3.537mins. The % purity of Sitagliptin and Simvastatin was found to be 99.8%. The system suitability parameters for Sitagliptin and Simvastatin 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)). The linearity study n Sitagliptin and Simvastatin was found in concentration range of 6µg-14µg and 18µg-42µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 100.351% and 100.93%, %RSD for repeatability was 0.177 and 0.595. The precision study was precise, robust, and repeatable. 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.

REFERENCES

1. Kealey D, Haines PJ. *Analytical Chemistry.* 1st ed. Oxford: BIOS Scientific Publishers; 2002. p. 1-7.
2. Braithwaite A, Smith FJ. *Chromatographic Methods.* 5th ed. Dordrecht: Kluwer Academic Publishers; 1996. p. 1-2.
3. Weston A, Brown PR. *HPLC Principle and Practice.* 1st ed. San Diego: Academic Press; 1997. p. 24-37.
4. Kazakevich Y, Lobrutto R. *HPLC for Pharmaceutical Scientists.* 1st ed. Hoboken: Wiley-Interscience; 2007. p. 15-23.
5. Chromatography [Internet]. Wikipedia; 2023 [cited 2025 Sep 4]. Available from: http://en.wikipedia.org/wiki/Chromatography
6. Meyer VR. *Practical High-Performance Liquid Chromatography.* 4th ed. Chichester: John Wiley & Sons; 2004. p. 7-8.
7. Sahajwalla CG. *New Drug Development.* Vol. 141. New York: Marcel Dekker Inc.; 2004. p. 421-6.
8. Shewiyo DH, Kaale E, Risha PG, Dejaegher B, Verbeke JS, Heyden YV. Development and validation of a liquid chromatographic method for analysis of rifampicin and pyrazinamide in fixed-dose combination products. *J Pharm Biomed Anal.* 2012;66:11-23. doi:10.1016/j.jpba.2012.03.037
9. United States Pharmacopeia. General Tests, Chapter 621 – Chromatography System Suitability. *USP 31.* Rockville, MD: USP Convention; 2009.
10. FDA. Guidance for industry: Analytical procedures and method validation – Chemistry, manufacturing, and controls documentation. Rockville, MD: CDER & CBER; 2000.
11. Korany MA, Mahgoub H, Ossama TF, Hadir MM. Application of artificial neural networks for response surface modelling in HPLC method development. *J Adv Res.* 2012;3(1):53-63. doi:10.1016/j.jare.2011.02.007
12. Swartz ME, Jone MD, Fowler P, Andrew MA. Automated HPLC method development and transfer. *LC GC N Am.* 2002;75:49-50.
13. Snyder LR, Kirkland JJ, Glajch JL. *Practical HPLC Method Development.* 2nd ed. New York: John Wiley & Sons; 1997. p. 643-712.
14. Swartz M, Murphy MB. New frontiers in chromatography. *Am Lab.* 2005;37(19):22-7.
15. Dolan JW. Peak tailing and resolution. *LC GC N Am.* 2002;20:430-6.
16. Chan CC, Lee YC, Lam H. *Analytical Method Validation and Instrument Performance Verification.* Vol. I. Hoboken: Wiley-Interscience; 2004.
17. Shabir GA, Lough WJ, Arain SA, Bradshaw TK. Evaluation and application of best practice in analytical method validation. *J Chromatogr A.* 2007;1158(1-2):311-33. doi:10.1016/j.chroma.2007.05.094
18. Miller JN, Miller JC. *Statistics and Chemometrics for Analytical Chemistry.* 5th ed. Harlow: Pearson Prentice Hall; 2005. p. 263.
19. Huber L. Validation of HPLC methods. *BioPharm Int.* 1999;12:64-6.
20. European Commission. Quality control procedures for pesticide residues analysis. Report SANCO/10232/2006. Brussels: Directorate-General for Health and Consumer Protection; 2006.
21. International Conference on Harmonisation (ICH). Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva: ICH Secretariat, IFPMA; 2005.
22. DrugBank. Simvastatin [Internet]. 2023 [cited 2025 Sep 4]. Available from: https://www.drugbank.ca/drugs/DB00641
23. PubChem. Simvastatin [Internet]. 2023 [cited 2025 Sep 4]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Simvastatin
24. Wikipedia. Simvastatin [Internet]. 2023 [cited 2025 Sep 4]. Available from: https://en.wikipedia.org/wiki/Simvastatin
25. DrugBank. Sitagliptin [Internet]. 2023 [cited 2025 Sep 4]. Available from: https://www.drugbank.ca/drugs/DB01261
26. PubChem. Sitagliptin [Internet]. 2023 [cited 2025 Sep 4]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Sitagliptin
27. Wikipedia. Sitagliptin [Internet]. 2023 [cited 2025 Sep 4]. Available from: https://en.wikipedia.org/wiki/Sitagliptin
28. Arige SS, Arige SD, Rao AL. Development and validation of sitagliptin and simvastatin tablets by using RP-HPLC method. *Int J Appl Pharm Sci.* 2017;4(1):36-43.
29. Patil PM, Rathod SD, Chopade VV. Development and validation of RP-HPLC method for the estimation of sitagliptin phosphate and simvastatin in bulk and pharmaceutical dosage form. *Int J Pharm Pharm Res.* 2015;5(1):90-100.

30. Chavan V, Ghante M, Sawant S. Development and validation of RP-HPLC method for simultaneous estimation of sitagliptin phosphate and simvastatin in bulk and tablet dosage form. *J Appl Pharm.* 2014;6(3):327-38.
31. Sreelakshmi V, Rao VU, Pugazhendhy S, Shrivastava SK, Sunitha M. Development and validation of simvastatin by RP-HPLC method in bulk drug and pharmaceutical dosage forms. *Am J PharmTech Res.* 2013;3(5):1-8.