***Original Research Article***

# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN IN BULK AND TABLET DOSAGE FORM

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## ABSTRACT

A simple and new Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed for the simultaneous estimation of Sitagliptin phosphate and Simvastatin in bulk and tablet dosage form. Separation was achieved with aHi-Q Sil $C_{18}$(250 mm × 4.6 mm, 5 µm Particle size) column at ambient temperature in isocratic mode withmobile phase containing acetonitrile, methanol and 10 mM phosphate buffer (65:25:10 % v/v/v) pH 4 adjusted with orthophosphoric acid, pumped at flow rate of 1.2 ml/min and eluent was monitored at250 nm. The selected chromatographic conditions were found to be effectively separate Sitagliptin phosphate and Simvastatin with retention time of 2.2 and 6.8 min respectively. The proposed method was validated as per ICH guidelines for linearity, precision, accuracy, LOD and LOQ. Both the drugs found to be linear within the conc. range of 100-600 and 20-120 µg/ml for Sitagliptin phosphate and Simvastatin respectively. The results of validation parameters indicates that the proposedmethod was also found to be accurate, precise, robust and sensitive. It can also beused for routine quality-control analysis of these drugs in combination tablets.

***Keywords*:** RP-HPLC, Sitagliptin phosphate (STG), Simvastatin (SMV) and ICH guidelines

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## INTRODUCTION

Sitagliptin phosphate (STG) is the first of a new class of drugs i.e. oral dipeptidyl peptidase-4 (DPP-4) inhibitor for the treatment of type II diabetes which improves glycaemic control by inhibiting DPP-4 inactivation of the increatin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP). This increases active increatin and insulin levels and decreases glucagon levels and post-glucose-load glucose excursion. Chemically it is known as (2R)-1(2,4,5-triflourophenyl)-4-oxo-4-[3(trifluoromethyl)-5,6-dihydro[1,2,4]-triazolo-[4,3-a]-pyrizin-7(8H)-yl]butan-2-amine (Fig. 1) [1]. Sitagliptin phosphate can be estimated by

Different analytical techniques such as UV spectrophotometry [2-8], RP-HPLC [8-13], HPTLC [13], LC-MS [14-17] and capillary zone electrophoresis [18] alone or in combination with other agents.

Simvastatin is one of the well-known HMG-COA reductase inhibitorbelonging to the class of statins. It act by inhibiting HMG-CoA reductase, a rate limiting enzyme in the synthesis of cholesterol in liver and used for the treatment of dyslipidemia and the prevention of cardiovascular diseases [19]. It is chemically known as (1, 3, 7, 8, 8a) -8-{2-[(2r, 4r)-4-hydroxy-6oxotetrahydro2H-pyran-2yl] ethyl}-3, 7-dimethyl-1, 2, 3, 7, 8.8a-hexahydronaphthalen-1-yl-2, 2-dimethyl butanoate (Fig.1). A HMG-CoA reductase (3-hydroxy-3-methylglutarylcoenzyme), the rate limiting enzyme of the HMG-CoA reductase pathway, the metabolic path way responsible for the endogenous production of cholesterol. Simvastatin is prodrug which is converted into its β- hydroxy form which inhibits HMG CoA Reductase enzyme, a rate limiting enzyme in the synthesis of cholesterol in liver [20].

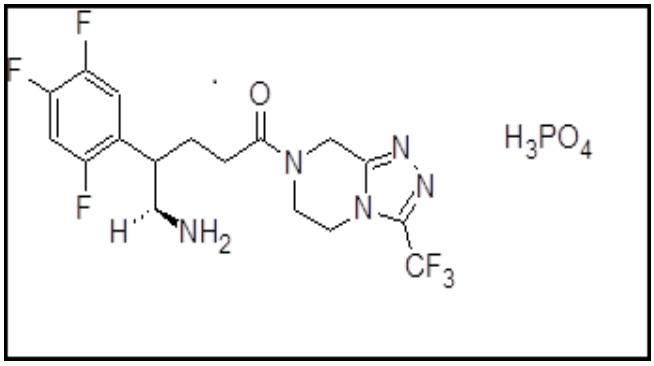


**Figure 1** Chemical Structure of Sitagliptin phosphate

The drug is officially listed in US pharmacopeia, British pharmacopeia and European pharmacopeia. Simvastatin can be estimated by UV spectrophotometry [21-33],RP-HPLC[32-51], HPTLC[52-56]and LC-MS/MS [57] alone or in combination with other drugs.

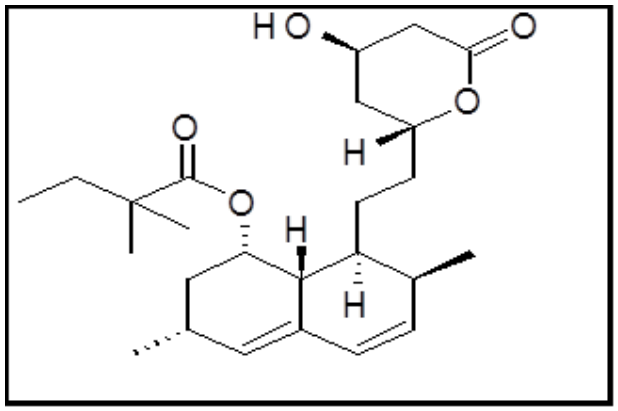


**Figure 2** Chemical Structure of Simvastatin

Literature survey reveals that so far, many RP-HPLC methods have been reported for the estimation of Sitagliptin phosphate and Simvastatin with alone or in combination with each other or with other drugs. But most of the methods included acetonitrile and different buffers as the part of their mobile phase [12-19] and [35-52]. Therefore the main objective of the proposed method was to develop simple,new accurate, precise, sensitive and robust RP-HPLC method for the simultaneous estimation of Sitagliptin phosphate and Simvastatin in bulk and tablet dosage form and validate as per ICH guidelines [57].

## MATERIALS AND METHODS

### Chemicals and Reagents

The pure API samples of Sitagliptin phosphate and Simvastatin were obtained as free gift samples from Getz Pharma Pvt. Ltd; Mumbai and Gen Pharma International Pvt. Ltd; Pune respectively while all solvents such as methanol, acetonitrile and ortho-phosphoric acid used were of HPLC grade (Thomas Baker, India) and double distilled water was used for whole study. The marketed combined pharmaceutical dosage form of Sitagliptin phosphate (100 mg) and Simvastatin (20 mg) i.e. **Juvisync (MSD India)** was purchased from local market.

### Instrumentation

The method was developed on Jasco HPLC instrument equipped with quaternary gradient pump Jasco PU-2089 Plus, Photo Diode Array (PDA) Detector MD-2018 Plusand Hi-Q Sil octadecyl column (250 mm × 4.6 mm, 5 µm particle size) and operated with ChromNAV software.

### Chromatographic conditions

TheHi-Q Sil $C_{18}$(250 mm × 4.6 mm, 5 µm Particle size) column was used at ambient temperature. The mobile phase consists of acetonitrile, methanol and phosphate buffer (65:25:10 % v/v/v) of pH 4 adjusted with ortho-phosphoric acid, pumped at flow rate of 1.2 ml/min, degassed by sonication and then filtered through a Nylon 0.2 µ membrane filter before use. The elution was monitored at 250 nm with the help of PDA detector and injection volume was 20 µl.

### Preparation of standard stock solutions

25 mg of Sitagliptin phosphate and 2.5 mg of Simvastatin was accurately weighed and transferred individually in separate 25 ml of volumetric flask and dissolved first in 15 ml of mobile phase, sonicated for 15 minutesand then final volume made upto 25 ml with mobile phase to form std. stock solutions of 1000 µg/ml of Sitagliptin phosphate and 100 µg/ml of Simvastatin and filtered through 0.45 µ filter paper. These stock solutions were further diluted to form working stock solutions.

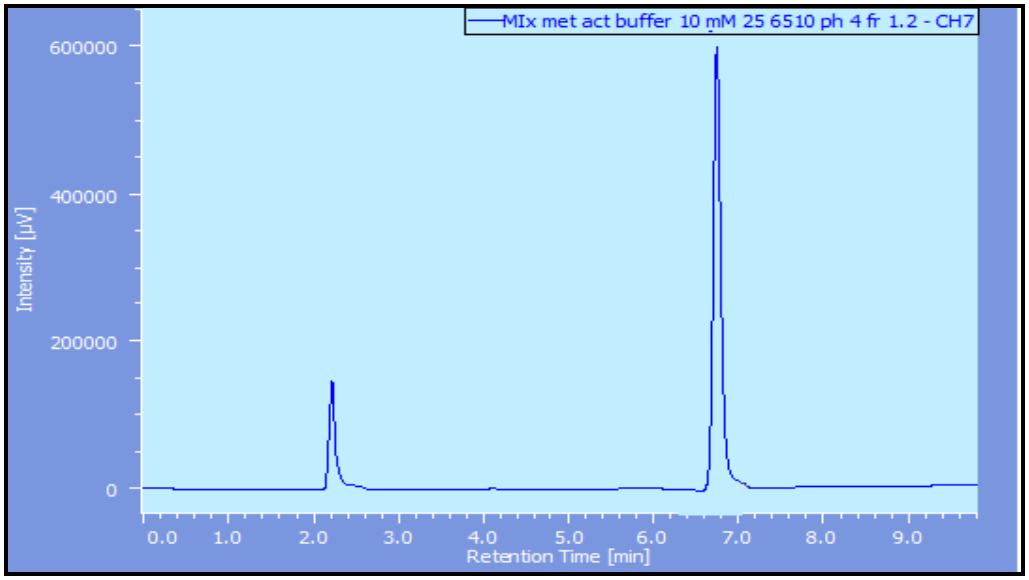


**Figure 3:** Chromatogram of mixture of STG and SMV in developed mobile phase

### Sample preparation

Twenty tablets were accurately weighed, finely powdered and average weight calculated. Powder weight equivalent to 100 mg of Sitagliptin phosphate and 20 mg of Simvastatin was weighed and transferred in separate 100 ml volumetric flask and dissolved in sufficient quantity of mobile phase, sonicated for 15 minutes, properly shaken,filtered through 0.45 µ Whatman filter paper and then final volume made upto 25 ml using mobile phase to form 1000 µg/ml of Sitagliptin phosphate and 200 µg/ml of Simvastatin. 1ml aliquot of this stock solution is then diluted upto 10 ml using mobile phase which was then eluted on RP-HPLC instrument under optimized chromatographic conditions to determine Sitagliptin phosphate and Simvastatin in Tablet dosage form.

### Method Validation

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for its intended use.Validation of developed HPLC method was carried out as per International conference of Harmonization (ICH guidelines) $Q_2$ ($R_1$) for linearity, precision, accuracy, LOD and LOQ. [58]

#### *Linearity*

From std. 1000µg/ml stock solution of Sitagliptin phosphate and 100 µg/ml stock solution of Simvastatin, pipette out aliquots of 1 to 6 ml of STG & 2 to 12 ml of SMV and transferred to series of 10 ml volumetric flasks and final volume made upto mark with mobile phase to form solutions of 100 to 600µg/ml of STG and 20-120 µg/ml of SMV. These solutions were then eluted at optimized chromatographic conditions at respective λmax and then calibration curve was plotted as peak area vs. concentration to check the linear relationship between peak area and concentration of Sitagliptin phosphate and Simvastatin [1].

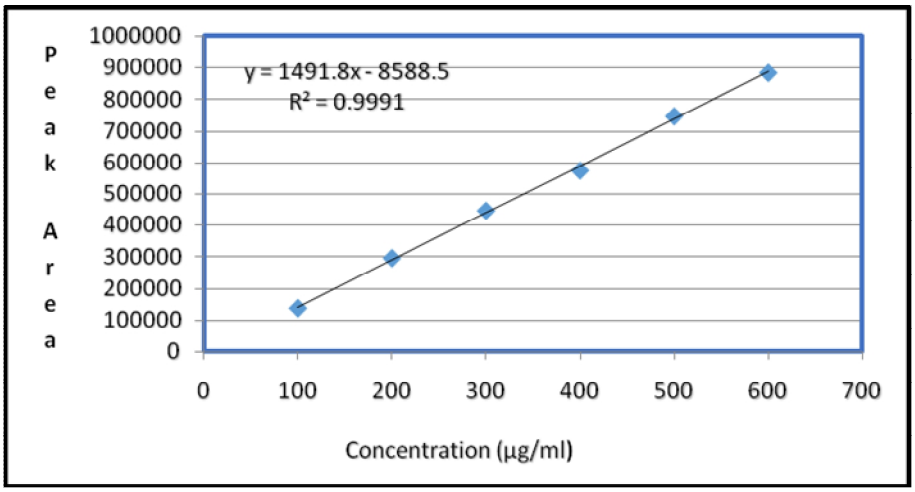


**Figure 4:** Calibration curve of Sitagliptin phosphate

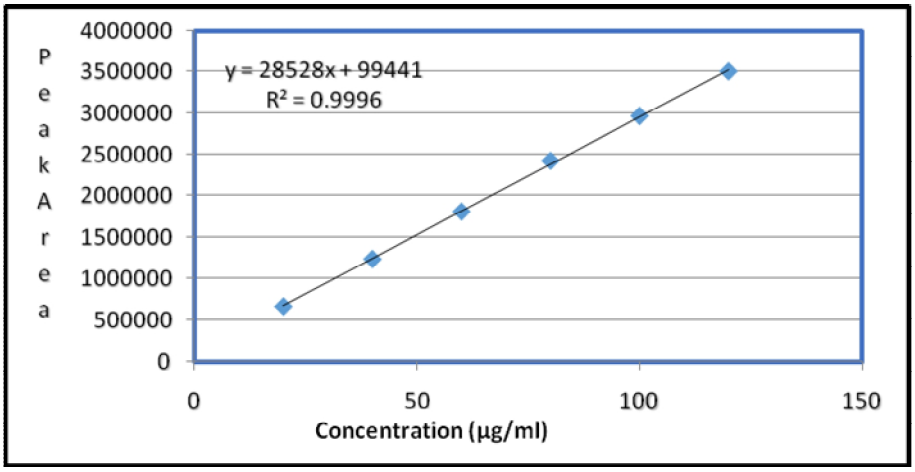


**Figure 5:** Calibration curve of Simvastatin

### *Precision*

Precision study expressed by carrying out Repeatability (intraday precision) and interday precision. The intraday (Repeatability) and interday precision study were carried out by estimating corresponding responses three times on the same day (Intraday) and on the three different days (Interday) for the three different concentrations (100, 200 and 300µg/ml) of STG and (20,40 and 60µg/ml ) for SMV. The results of precision study were reported in terms of% relative standard deviation [1].

### *Accuracy*

To carry out accuracy study of proposed method, the recovery studies were carried out by standard addition method at three different levels (80, 100 and 120 %) of API sample of Sitagliptin phosphate

and Simvastatin to the previously analysed solution of formulation containing 200µg/ml of STG and 40 µg/mlof SMV [1]. The results of precision study were carried out in terms of % RSD.

***LOD and LOQ***

Limit of detection (LOD) is defined as lowest concentration of analyte that can be detected while limit of quantitation is defined as lowest concentration of analyte that can be quantitated with suitable precision and linearity. LOD and LOQ can be calculated from the following formulas

$$LOD = 3.3* r / S \quad \text{and} \quad LOQ = 10 * r/ S$$

Where r is the Standard deviation of y-intercept of the regression line and S is slope of the calibration curve [1].

***Robustness***

Robustness of developed RP-HPLC method was studied by effect on retention time of STG and SMV bychanging flow rate (± 0.1 ml/min), composition of organic phase (± 1 %) and pH of mobile phase (±0.1).

**RESULTS AND DISCUSSION**

**Method Development**

As literature survey already reveals that there were so many RP-HPLC methods have been reported for the estimation of STG and SMV alone or in combination with each other or with other drugs which included acetonitrile and different buffers as part of their mobile phase, therefore initially we started to develop method by using acetonitrile and methanol in different compositions. Butproblem encountered with the shape of Sitagliptin phosphate, therefore we adjusted pH with the help of ortho-phosphoric acid at 4 and add 10 mM phosphate buffer to get low tailing factor & sharp peak of STG and finally after many trials, we optimized acetonitrile:methanol:10 mM phosphatebuffer (65:25:15 % v/v/v) of pH 4 as mobile phase.

**Method Validation**

***Linearity***

Linearity was evaluated by analysis of working standard solution of Sitagliptin phosphate and Simvastatin at six different concentrations. STG and SMV were found to be linear within conc. range of 100-600 µg/ml and 20-120 µg/ml with regression coefficient of 0.9991 & 0.9998 respectively. The results of regression analysis are summarized in (Table 1). Results shows that within the concentration range mentioned above, there was an excellent correlation between peak area and concentration of STG and SMV (Fig 4 and 5) [1].

**Table 1.Results of regression analysis of STG and SMV**

| Drugs | Beer's Range (ng/band) | Regression equation | Regression coefficient ($r^2$) | λmax (nm) |
|---|---|---|---|---|
| **Sitagliptin phosphate** | 100-600 | y = 1491.8x -8588.5 | 0.9991 | 267 |
| **Simvastatin** | 20-120 | y = 2852x + 99441 | 0.9998 | 237 |

***Precision***

The repeatability (intra-days precision) is expressed as percentage relative standard deviations (% RSD) for the STG at the concentration of 100, 200 and 300 µg/ml and their average % RSD value was0.278 while for thetime-different intermediate precision (inter-days precision) of the STG at the same concentrations,the% RSD values was 0.198respectively. The % RSD for the SMV at the

concentration of 20, 40 and 60 µg/ml and their average % RSD value was 0.476 while for thetime-different intermediate precision (inter-days precision) of the SMV at the same concentrations, the% RSD values was 0.455respectivelyThe % RSDlevels of intra-day and inter-day precision were less than 2.0in all cases, which indicated that the method found to be precise and there were no significant variations in the analysis of sitagliptin phosphate and simvastatin and therefore the present RP-HPLC method was found to be precise [1]. The results of precision study were summarized in (Table 2 and 3).

**Table 2. Result of Intraday (Repeatability) Precision studies**

| Drugs | Conc. taken (µg/ml) | Conc. found * (µg/ml) | % Amt. found | S.D. | % R.S.D. |
|---|---|---|---|---|---|
| **STG** | 100 | 99.91 | 99.91 | 0.277 | 0.278 |
| | 200 | 199.58 | 99.79 | | |
| | 300 | 298.14 | 99.38 | | |
| **SMV** | 20 | 19.96 | 99.84 | 0.476 | 0.476 |
| | 40 | 39.92 | 99.81 | | |
| | 60 | 60.39 | 100.65 | | |

***Average of three estimations, S.D- Standard Deviation, % R.S.D.- % Relative Standard Deviation**

**Table 3. Result of Interday Precision studies**

| Drugs | Conc. taken (µg/ml) | Conc. found * (µg/ml) | % Amt. found | S.D. | % R.S.D. |
|---|---|---|---|---|---|
| **STG** | 100 | 99.75 | 99.75 | 0.197 | 0.198 |
| | 200 | 99.30 | 99.36 | | |
| | 300 | 299.13 | 99.71 | | |
| **SMV** | 20 | 19.93 | 99.68 | 0.455 | 0.455 |
| | 40 | 39.94 | 99.85 | | |
| | 60 | 60.32 | 100.54 | | |

*** Average of three estimations**

**Table 4: Results of accuracy (Recovery) studies.**

| Recovery Level (%) | Drug | Conc. of drug (µg/ml) | | % Total Amt. of Drug Found* | S.D | % R.S.D. |
|---|---|---|---|---|---|---|
| | | From Tablet | From API | | | |
| **80** | **STG** | 200 | 160 | 99.42 | 0.28006 | 0.436616 |
| **100** | | 200 | 200 | 99.98 | | |
| **120** | | 200 | 240 | 99.69 | | |
| **80** | **SMV** | 40 | 32 | 99.55 | 0.280912 | 0.436427 |
| **100** | | 40 | 40 | 100.38 | | |
| **120** | | 40 | 48 | 100.2 | | |

*** Average of three estimations.**

***Accuracy***

The accuracy was assessed by the standard addition method of three replicate determinations of three different solutions containing 160, 200 and 240 µg/ml of STG and 32, 40 and 48 of SMV. The average % recoveries for three different concentrations were found to be 98.69 %for STG and 100.04 % for SMV respectively using proposed RP-HPLC method. The higher values indicate that the proposed method was found to be accuratefor the determination of STG and SMV in pharmaceutical dosage form [1]. Results of recovery studies are summarized in (Table 4).

***LOD and LOQ***

The limit of detection were found to be 51.80µg/ml &6.80µg/ml for Sitagliptin phosphate and Simvastatin respectively while limit of quantification were found to 156.06µg/ml& 20.61µg/mlfor Sitagliptin phosphate and Simvastatin respectively by proposed RP-HPLC method [1]. Results of LOD and LOQ are summarized in (Table 5).

**Table 5. Results of LOD and LOQ**

| Parameter | Sitagliptin Phosphate | Simvastatin |
|---|---|---|
| **LOD (µg/ml)** | 51.50 | 6.80 |
| **LOQ (µg/ml)** | 156.06 | 20.61 |

***Robustness***

The results and the experimental range of the selected variablesevaluated in the robustness assessment shown in (Table No. 6).There were no significant changes in the chromatographic pattern when the modifications were made in the experimental conditions, thus showing the method to be robust.

**Table 6: Results of Robustness studies**

| Factor | Level | Sitagliptin phosphate (Retention Time) | Simvastatin (Retention Time) |
|---|---|---|---|
| | | **A: Flow rate (ml/min)** | |
| **1.1** | **- 0.1** | 2.4 | 7.2 |
| **1.2** | **0** | 2.2 | 6.8 |
| **1.3** | **+ 0.1** | 1.9 | 6.1 |
| | | **B: Percentage of Acetonitrile in the mobile phase (v/v)** | |
| **64** | **- 1** | 2.2 | 6.7 |
| **65** | **0** | 2.2 | 6.8 |
| **66** | **+ 1** | 2.2 | 6.9 |
| | | **C: pH of mobile phase** | |
| **3.9** | **- 0.1** | 2.1 | 6.8 |
| **4.0** | **0** | 2.2 | 6.8 |
| **4.1** | **+ 0.1** | 2.2 | 6.8 |

**System Suitability parameters**

System suitability test was carried out to evaluate resolution and reproducibility of the system for the analysis to be performed, using five replicate injections of reference solution containing 200 µg/ml STG

and 40 µg/ml SMV. The parameters measured were peak area, retention time, theoretical plates and tailing factor. The results of system suitability parameters are summarized in (Table 7)

**Table 7: System suitability parameters**

| Sr. No. | System suitability parameters | Sitagliptin Phosphate | Simvastatin |
|---|---|---|---|
| 1 | **Retention time** | 2.2 | 6.8 |
| 2 | **Resolution** | 12.81 | |
| 3 | **No. of Theoretical plates** | 6899 | 3327 |
| 4 | **Tailing factor** | 1.9 | 1.85 |

**Assay**

Analysis of sample of marketed tablet containing 100 mg Sitagliptin phosphate and 20 mg Simvastatin was carried out andthe amounts recovered were expressed as a percentage amount ofthe label claims. The percentage recovery of Sitagliptin phosphate and Simvastatin were 99.40and99.46 respectively, andthese values are complying with the assay specifications for active drug (Simvastatin) in the United States of Pharmacopoeia (90.0–110.0%)which are required to be met by most drug formulations [1]. Results of tablet assay are summarized in (Table 8)

**Table 8: Results of Tablet assay**

| Active Ingredients | Label Claim (mg/tab) | Amount of Drug* Estimated (mg/tab) | % Assay |
|---|---|---|---|
| **Sitagliptin phosphate** | 100 mg | 99.40 | 99.40 |
| **Simvastatin** | 20 mg | 19.46 | 99.46 |

***Average of six estimations**

**Method application**

The proposed RP-HPLC method was applied for the determination of Sitagliptin phosphate and Simvastatin in tablet dosage forms, without prior separation of the excipients of the formulation. The results demonstrate the quality of the analyzed pharmaceutical samples and the applicability of the method for QC analysis.

## CONCLUSIONS

A simple andnew RP-HPLCmethod have been developed for the simultaneous determination of Sitagliptin phosphate and Simvastatin in bulk and tablet dosage formand validated as per ICH guidelines. The results of the validation studies proved that the proposed RP-HPLC method was also accurate, precise, specific, robust and sensitive. It possessed significant linearity, precision, high efficiency and resolution and no interference from the excipients.The proposed method was successfully applied and can be suggested for the quantitative analysis of Sitagliptin phosphate and Simvastatin in pharmaceutical formulations for QC, where economy and time areessential and to assure therapeutic efficacy.

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