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Simultaneous Estimation of Sitagliptin Phosphate Monohydrate and Simvastatin in Bulk and Tablet Dosage Form by RP-HPLC

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ABSTRACT

A new, simple, accurate, precise and reproducible RP-HPLC method for the simultaneous estimation of Sitagliptin Phosphate Monohydrate and Simvastatin has been developed and validated. Chromatographic separation was achieved using Neosphere C-18 column (4.6 mm × 250mm,5mm) in gradient mode with a mobile phase consisting a mixture of acetonitrile:methanol:0.1% orthophosphoric acid in water (70:15:15v/v) at a flow rate of 1ml/min. The eluents were detected at 254 nm using UV detector. The retention time of Sitagliptin Phosphate Monohydrate and Simvastatin were found to be 2.09min and 7.79min respectively. The method was linear over the concentration range of 25-150µg/mL for Sitagliptin Phosphate Monohydrate and 10-60µg/mL for Simvastatin. The % recoveries for Sitagliptin Phosphate Monohydrate and Simvastatin were found to be 98.98-101.06% and 99.89-102.43% respectively. The developed method was validated for parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness and robustness as per International Conference on Harmonization guidelines and the results were found to be within the limits. This validated method can be used for the routine quality control analysis of Sitagliptin Phosphate Monohydrate and Simvastatin in combined dosage form.

Keywords: Sitagliptin phosphate monohydrate, Simvastatin, Reverse-phase high performance liquid chromatography, Simultaneous estimation, Validation.

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INTRODUCTION

Sitagliptin phosphate monohydrate (SPM) 7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-[3-(trifluoromethyl)-1,2,4-triazolo[4,3-α]pyrazine phosphate monohydrate. SPM is a inhibitor of dipeptidyl peptidase-4 (DPP-4) called as gliptins, offer a new approach in the management of type 2 diabetes by inhibiting the enzyme DPP-4, which degrades gut-derived hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These hormones are released from the intestine during a meal and act on the pancreas to increase nutrient-stimulated insulin secretion. Several methods are available for the estimation of SPM as a single and combined dosage forms with other drugs. The determination of SPM has been carried out in tablet by RP-HPLC, HPTLC, UV Spectrophotometry, UPLC, tandem mass spectrometry, capillary electrophoresis.

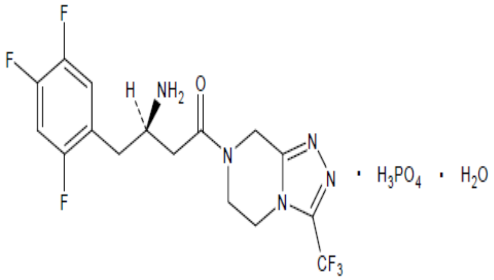

Figure 1: Sitagliptin phosphate monohydrate

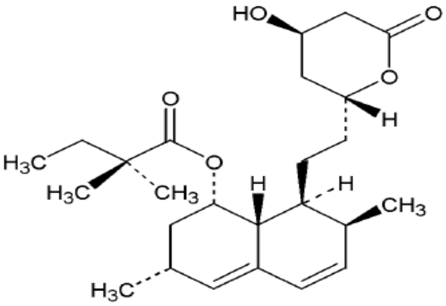

Figure 2: Simvastatin

Simvastatin (SMV) [1S-[1α,3α,7β,8β(2S*,4S*),8αβ]]-1,2,3,7,8,8a-Hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphthalenyl-2,2dimthylbutanoate.

Simvastatin is an inactive lactone which hydrolyzed in the liver to the corresponding active β-hydroxyacid form which has a potent activity in inhibiting HMG-CoA reductase. This enzyme catalyses the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in the biosynthesis of cholesterol. It is a lipid-lowering agent that reduces concentrations of total cholesterol, low-density lipoprotein, very low-density lipoprotein and plasma triglycerides, while elevating concentrations of high-density lipoprotein. Several methods are available for the estimation of SMV as a single and combined dosage forms with other drugs. The determination of SMV has been carried out in tablet by RP-HPLC, HPTLC, UV Spectrophotometry, UPLC, tandem mass spectrometry, capillary electrophoresis. The literature review revealed that there is no chromatographic method available for the simultaneous estimation of this combination. Hence an attempt was made to develop a validated analytical method for the simultaneous estimation of SPM and SMV combined dosage form using RP-HPLC method.

MATERIALS AND METHODS

Solvents and Chemical used

Acetonitrile HPLC grade**,** Methanol HPLC grade, Water HPLC grade**,** Orthophosphoric acid AR grade were procured from Merck chemicals, Mumbai. Sitagliptin phosphate was procured from Dr. Reddy's Laboratories, Hyderabad while Simvastatin was provided by Hetero Labs Ltd. Andhra Pradesh.

Instrument and Chromatographic conditions

Table 1: Chromatographic conditions

Parameters	**Specifications**
Instrument	Cyberlab,LC-100 B complete binary gradient HPLC systems with UV- VIS detector
Column	Neosphere C-18 (4.6 mm × 250 mm,5mm)
Mobile phase	Acetonitrile:Methanol:Water(70:15:15v/v/v) with 0.1% orthophosphoric acid
Flow rate	1ml/min
Detection wavelength	254 nm
Injection volume	20 µl
Mode of separation	Reverse phase
Run time	10min

Preparation of mobile phase

HPLC grade Acetonitrile, Methanol, Water in the ratio 70:15:15v/v was taken with 0.1% orthophosphoric acid and filtered by 0.45µm membrane filter. Then sonicated it for 15 mins

Preparation of standard stock solution:

Accurately weighed 25mg each SPM and SMV were separately transferred to two 25ml volumetric flasks, dissolve and make up the volume with mobile phase to obtain a concentration of 1000µg/ml of SPM and SMV respectively. From the resulting SPM stock solution pipette out 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml and transferred to 10ml volumetric flasks, and make up the volume with mobile phase. From 1000µg/ml of SMV stock solution pipette out 2.5ml and transferred to 25ml volumetric flask, and make up the volume with mobile phase to obtain a concentration of 100µg/ml of SMV. From the resulting stock solution pipette out 1ml, 2ml, 3ml, 4ml, 5ml, 6ml and transferred to 10ml volumetric flasks, and make up the volume with mobile phase.

Selection of analytical wavelength

From the standard stock solution, further dilutions were prepared using mobile phase. By appropriate dilution of standard stock solution, sample stock solution of suitable concentration of Sitagliptin Phosphate Monohydrate and Simvastatin were prepared separately. The standard solutions of 20µg/ml of Simvastatin and 50µg/ml of Sitagliptin phosphate monohydrate were then scanned in the UV spectrophotometer from 400-200 nm. In order to obtain accurate and reproducible results, the wavelength selected should be such that absorptivity of both the drugs should be as large as possible. At 254 nm wavelength get optimum absorption.

Preparation of standard calibration curve and selection of analytical concentration range

For SPM and SMV appropriate aliquots of standard stock solution were transferred to a series of 10ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain working standard solution of concentration of 25-150µg/ml for SPM and 10-60µg/ml for SMV. Six sets of each concentration of the drug were prepared. The standard calibration curves of peak area Vs concentration were plotted using the mean of these six observations. The concentration range over which the drugs were obeyed Beer-Lambert's law was found to be between 25-150µg/ml for SPM and 10-60µg/ml for SMV.

Analysis of Reference Standard

The stock solution containing mixture of SPM and SMV in ratio of 2.5:1 was taken, different dilution from stock solution were prepared having concentration range 25-150µg/ml for SPM and 10-60µg/ml for SMV. The middle range 100µg/ml for SPM and 40µg/ml for SMV were selected as a reference standard. Reference standard solution was injected to get the chromatogram.

Analysis of Tablet Dosage form

Tablet powder equivalent to 50mg of SPM and 20mg of SMV were weighed accurately and transferred in a 50ml volumetric flask, 30ml mobile phase was added to dissolve the drugs, then solution was sonicated for 20 mins for complete dissolution. The solution was diluted up to the mark to get 1000µg/ml of SPM and 400µg/ml of SMV. Filter the solution through Whatman filter paper no.41. From resulting stock solution pipette out 1ml and transferred to 10ml volumetric flask, and make up the volume with mobile phase to obtain a concentration of 100µg/ml SPM and 40µg/ml SMV. The resulting stock solution was filtered using 0.22µ nylon filter and 20µl solution was injected and chromatogram was recorded and the amount of drug was calculated.

METHOD VALIDATION

Linearity

The linearity of the analytical procedure is its ability (within a given range) to obtain the test results which are directly proportional to the concentration (amount) of analyte in the sample.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of the y intercept and slope of calibration curve were used to calculate the LOD and LOQ. LOD calculated from the formula,

$$\text{LOD} = \frac{3.3\,\sigma}{S}$$

LOQ calculated from the formula,

$$\text{LOQ} = \frac{10\,\sigma}{S}$$

Where, σ = standard deviation of the curve

S = slope of the calibration curve

Accuracy

Recovery studies were carried out by applying the method to drug content present in formulation to which known amount of reference standard of SPM added 80µg/ml for 80%, 100µg/ml for 100%, 120µg/ml for 120%, and SMV added 32µg/ml for 80%, 40µg/ml for 100%, and 48µg/ml for 120%. The solution for recovery studies at 80%, 100%, 120% levels were prepared in the same manner as that of UV-Spectrophotometric studies except that the mobile phase was used as a solvent. The solutions were filtered through 0.2µm membrane filter paper and analyzed by RP-HPLC method. At each level of recovery three determinations were performed.

Precision

Repeatability

The repeatability of sample application and measurement of peak area of the drug was calculated by repeating the assay six times for each concentration.

Intermediate precision

The sample application and measurement of drug was calculated by repeating the assay six times for each concentration in the same day for intraday precision. The inter-day precision was obtained by assay of six sample sets on different days.

Specificity

Specificity is the ability to find and quantify the compound of interest also in the presence of other compounds. This means of chromatographic methods, that the analyte can be separated with sufficient resolution and that it can be directed with suitable instrument. The specificity of method was checked by comparing the peak of pure Sitagliptin Phosphate Monohydrate and Simvastatin with that of tablet dosage form containing other excipients along with pure drugs. Baseline should not give any peak at corresponding retention time, also should not produce a noise.

Ruggedness

Same experiment was carried out by different analyst.

Robustness

Small but deliberate variation was done in method parameter such as change in flow rate of mobile phase by ± 0.1 ml/min.

RESULT AND DISCUSSION

Method Development

The selected and optimized mobile phase was Acetonitrile: Methanol: Water (70:15:15 v/v/v) with 0.1% orthophosphoric acid at a flow rate of 1ml/min. UV detection was carried out at 254 nm (Figure 3: shows overlay spectra of SPM and SMV).

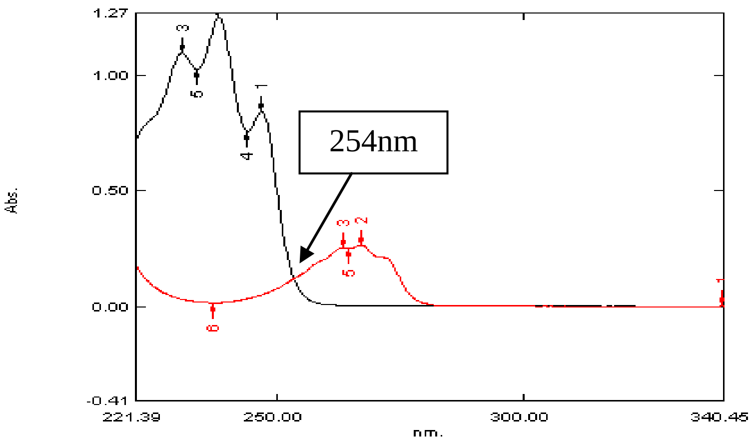

Figure 3: Overlay spectra of SPM and SMV showing at 254 nm

Analysis of Reference Standard:

The middle range 100µg/ml for SPM and 40µg/ml for SMV were selected as a reference standard.

Table 2: Analysis of Reference Standard of SPM and SMV

Sr. No	Name of drug	Concentration (µg/ml)	Retention time(min)	Peak area	Tailing factor	Theoretical plate
1	SPM	100	2.10	24443.2	1.50	6467.53
2	SMV	40	7.80	37572.45	1.30	13337.35

Analysis of Tablet Dosage form

Table 3: Analysis of Tablet Dosage form

Drug name	Amount taken (µg/ml)	Amount found (µg/ml)	Amount found (%)
SPM	100	98.24	98.24
SMV	40	41.12	102.8

Assay Limit: Simvastatin contains not less than 90.0% and not more than 110.0% of the stated amount of Simvastatin. Sitagliptin phosphate monohydrate contains not less than 98.0% and not more than 102.0% of the Sitagliptin.

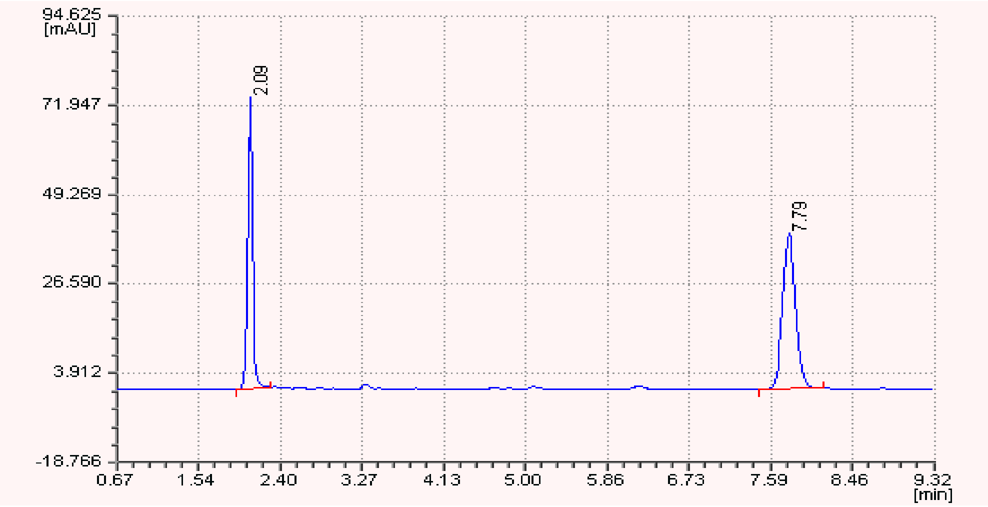

Figure 4: Typical chromatogram of Tablet dosage form shows Sitagliptin Phosphate Monohydrate at 2.09mins and Simvastatin at 7.79mins

METHOD VALIDATION

System Suitability

System Performance parameters of developed RP-HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N), tailing factor, resolution(R), retention time (RT) were determined. The results are shown in Table 4 it indicates good performance of system.

Table 4: System Performance for Sitagliptin phosphate and Simvastatin

Parameter	SPM	SMV
Theoretical plates	6467.53	13337.35
Tailing Factor	1.50	1.30
Retention time	2.09	7.84
Resolution		22.0

Linearity

The calibration curves were found to be linear in the concentration range of 25-150µg/ml for SPM and 10-60µg/ml for SMV. The standard working curve equation for SPM was found to be $y = 248.0x + 459.1$ with correlation coefficient value $r^2 = 0.9962$ and for SMV standard working curve equation was found to be $y = 908.7x + 587.3$ with correlation coefficient value $r^2 = 0.9985$.

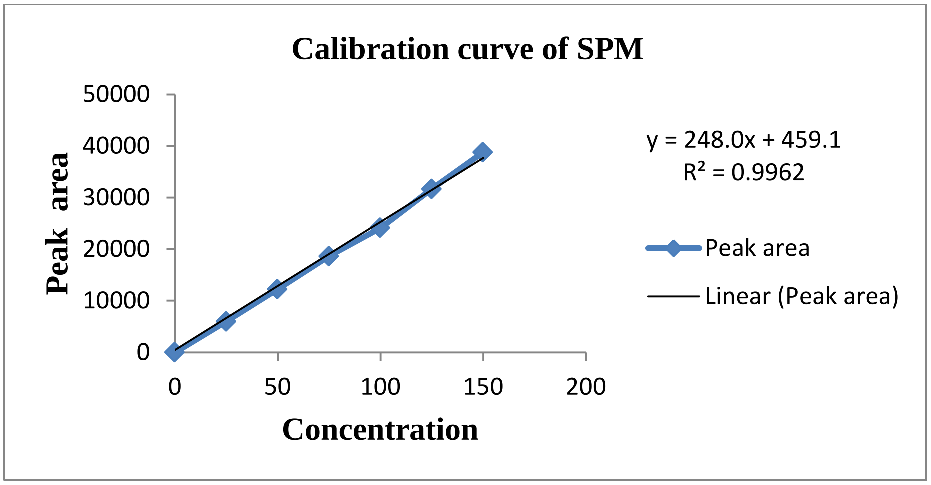

Figure 5: Standard calibration curve of SPM

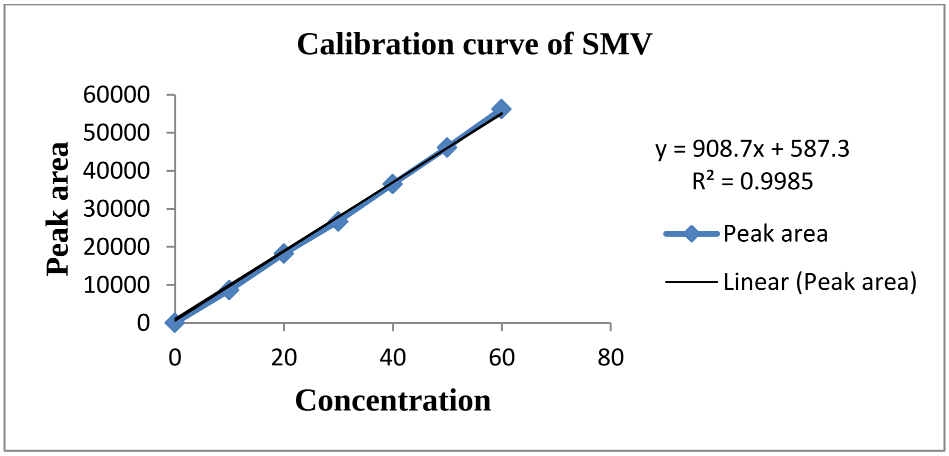

Figure 6: Standard calibration curve of SMV

Limit of detection and Limit of quantitation

It was calculated by standard deviation of the response and the slope of calibration curve

Table 5: LOD and LOQ

Parameter	SPM	SMV
L.O.D (µg/ml)	1.6254	0.9482
L.O.Q (µg/ml)	4.9254	2.8734

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for SPM and SMV

Table 6: Statistical validation of recovery studies

Level of Recovery (%)	% Mean recovery		% R.S.D	
	SPM	SMV	SPM	SMV
80	98.98	99.89	0.4575	0.8155
100	100.16	99.99	0.4136	1.3132
120	101.06	102.43	0.5910	1.3733

Precision

Repeatability

Table 7: Statistical validation of Tablet dosage form

Drug	Mean peak area	S.D	%R.S.D
SPM	24671.31	156.3	0.6336
SMV	36645.31	83.95	0.2291

Intermediate precision

Table 8: Inter-day and Intra-day Precision

Drug	Concentration (µg/ml)	Inter-day		Intra-day	
		Mean peak area	%RSD	Mean peak area	%RSD
SPM	100	25653.36	1.7533	24464.66	0.6479
SMV	40	36247.61	1.2790	36660.55	0.9546

Specificity

The chromatogram of formulation showed only two peaks at retention time of 2.09 and 7.79 mins of SPM and SMV respectively. Figure 4 indicating that there is no any interference from the excipients of formulation.

Ruggedness

Overall six results were obtained from two different Analyst were found to be within limits. Therefore, the HPLC method for the determination of both the drug is rugged. Limit: Overall RSD for six results obtained from two different analysts should not be more than 2.0%.

Robustness

Table 9: Robustness analysis of Tablet dosage form

Parameter	Level	% Assay		% RSD	
		SPM	SMV	SPM	SMV
Flow rate	0.9ml/min	99.22	100.7	1.11	0.83
	1.1ml/min	98.68	101.9	0.95	0.91

CONCLUSION

The RP-HPLC method was successfully developed and validated. The proposed method is simple, accurate, precise, and the statistical analysis proved that the method is reproducible and efficient for the simultaneous estimation of Sitagliptin phosphate monohydrate and Simvastatin as bulk drugs and in combined tablet dosage form without any interference from the excipients. From this study it was concluded that, the method can be employed for routine Quality control analysis.

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