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Development and Validation of RP-HPLC Method for the Estimation of Sitagliptin Phosphate and Simvastatin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, accurate rapid and precise RP-HPLC method has been developed and validated for determination of sitagliptin phosphate and simvastatin in bulk drug. The RP-HPLC separation was achieved on Grace C18 ACME 9000, C-18 (250 mm, 4.6 mm, 5µm) using mobile phase Methanol: Distilled Water (80:20), pH 4.2 adjusted with o-phosphoric acid with flow rate of 1.0 ml/min at ambient temperature. The retention times were 2.383 min. for sitagliptin and 7.000 min. for simvastatin. Calibration plots were linear over the concentration range 20-100µg/ml for sitagliptin and 10-50 µg/ml for simvastatin. The detection was carried out at a wavelength 254 nm. The method was validated statistically and applied successfully for the determination of sitagliptin and simvastatin. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for the routine determination of simvastatin in bulk drug.

1 INTRODUCTION

Sitagliptin chemically is (3R) -3-amino-1-[3- (trifluoromethyl)-6,8-dihydro-5h- [1,2,4] triazolo [3,4-c] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan- 1-one, structure shown in figure 1. Sitagliptin phosphate, an oral Anti-diabetic agent that blocks Dipeptidyl peptidase-4 (DPP-4) activity.Sitagliptin increased incretin levels (GLP-1 and GIP) which inhibit glucagon release, in turn decreases blood glucose, but more significantly increases insulin secretion [1-3].

Simvastatin (SIMVA) is chemically is 2,2-Dimethyl butanoic acid (1S,3R,7S,8S,8aR)-1,2,3,7,8,8ahexahydro-3,7-dimethyl-8-[2-[(2R,4R)- tetrahydro-4- hydroxy-6 oxo2H pyran-2yl]ethyl]1-napthalenyl ester ;structure of simvastatin shown in figure 2. Simvastatin used as a HMG-CoA reductase inhibitors Simvastatin is used in the treatment of primary hypercholesterolemia and is effective in reducing total and LDL-Cholesterol as well as plasma triglycerides and apolipoprotein B [4-6].

The literature reveals that there are some of the methods have been reported for sitagliptin UV, HPLC, and spectrophotometry [7-9]. For Simvastatin also have been reported UV, HPLC, and spectrophotometry [10-12]. As on Sitagliptin and Simvastatin one method was reported on spectrophotometry [13].This paper presents simple, rapid, accurate and economical methods for RP-HPLC method for the estimation of sitagliptin phosphate and simvastatin in bulk and pharmaceutical dosage form

2 MATERIALS AND METHODS

2.1 Instrument:

Different kinds of equipments like Analytical weighing balance, HPLC system (SHIMADZU-SPD 20A), Injector (Rheodyne,20µl), sonicator, pH meter, vaccum filter pump, Millipore filtration kit, mobile phase reservoir, Water bath, Sample filtration assembly and glassware's were used throughout the experiment.

2.2 Solvent used:

All the reagents used were of HPLC grade and analytical grade and were purchased from Merck Chemicals, India. Reference standard of Sitagliptin and Simvastatin was supplied as gift sample

from Merck Pharmaceutical Laboratories Limited, Sinner, Nasik(M.S.) India; with purity of 99.98%.

2.3 Preparation of Standard Stock Solution: Accurately weighed quantity of Sitagliptin phosphate equivalent to (10mg) and Simvastatin (10mg) were transferred into two separate 100 ml volumetric flasks. The drug was dissolve in 50 ml of mobile phase with shaking and sonicated for 10 min then volume made up to the mark with mobile phase to obtain standard stock solution of each drug of concentration 100 µg/ml. The stock solutions were filtered through a 0.45 µ membrane filter.

2.4 Sample preparation:

Transfer precise test portions of powdered tablets equivalent to 100mg of Sitagliptin and 40 mg of simvastatin in 100ml volumetric flask, add mobile phase and sonicate till dissolved with intermediate shaking for 10mins. Dilute to volume with mobile phase and mix. Filter a portion of the resulted solution and discard first few ml of the filtrate. Transfer 1.0ml of the above filtered solution into a 10ml volumetric flask, dilute to volume with mobile phase. UV Spectrum of sitagliptin and simvastatin at isobestic point 254nm and optimized chromatogram of sitagliptin and simvastatin by RP-HPLC; spectra shown in figure 3

2.4 Preparation of buffer

The pH of the solution was adjusted to 4.0 +0.01 with orthophosphoric acid and then filtered through 0.45µm membrane filter.

2.5 Preparation of Mobile Phase: Double distilled water pH 4.2 adjusted with o-phosphoric acid. The mobile phase consisting of Methanol: Distilled Water (80:20). The mobile phase prepared was then filtered through a 0.45 µ membrane filter.

2.6 Preparation of standard calibration curves and selection of analytical concentration ranges: For each drug, appropriate aliquots of standard stock solutions were transferred to a series of 10 ml volumetric flasks. The volume was made up to the mark with distilled water to obtain working standard solutions for each drug of concentrations of 10-50 µg/ml and 20-100 µg/ml. Three sets of each concentration of the drugs were prepared separately. The standard

calibration curves of Peak area Vs Concentration were plotted using the mean of these three independent observations. The concentration range over, which the drugs obeyed Beer - Lambert's law was found to be between 20 to 100 µg/ml for Sitagliptin and 10 to 50 µg/ml for Simvastatin respectively.Fig no.4 and 5 shows the standard calibration curves of SITA and SIMVA**.**

2.7Chromatographic conditions

UV Spectrum of sitagliptin and simvastatin at isobestic point 254nm shown in fig no. 3 hence; analysis was carried at 254nm using a Grace C18 ACME 9000, C18 reverse phase column of 250x 4.0mm i.d., 5µm dimensions at ambient temperature. The mobile phase consisted of Methanol: Distilled Water, pH 4.2 adjusted with o-phosphoric acid (80:20) that was set at a flow rate of 1.0ml/min.

Columns: Grace C18 ACME 9000 C18 5µm 4.6×250mm (i.d) column

Mobile phase: Methanol: Distilled Water, pH 4.2 adjusted with o-phosphoric acid

Isocratic: 80:20

Flow rate: 1.0 mL/min

Detector: UV, D2 lamp, 254 nm

Column temperature: Controlled room temperature (25°C)

Injection: 20 µL sample loop.

3 Method validations:

The proposed method was validated as per ICH guidelines. The dug solutions were prepared as per earlier adopted procedure given in the experiment.

3.1 Accuracy:

Recovery studies were carried out by applying the method of standard drug addition at 80 %, 100 % and 120 % levels.

3.2 Intermediate precision:

The system repeatability was determined by replicate injections of the prepared sample solutions. The peak areas for the drugs were noted by repeating the assay for each concentration on the same day for intraday precision. The inter-day precision was obtained by the assay of sample sets on different days.

3.3 Repeatability:

Repeatability was determined by analyzing 20 µg/ml and 8 µg/ml concentration of SITA and SIMVA solution for six times.

3.4 Specificity:

The specificity of the HPLC method was ascertained by analyzing standard drug and sample solutions.

3.5 Limit of Detection (LOD) and Limit of Quantitation:

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 the calibration curves were used to calculate the LOD and LOQ. The results of the same are shown in Table No. 26.

3.6 Robustness:

To evaluate the robustness of the developed method, deliberate variations were made in the method parameters such as change in the pH of the mobile phase, flow rate and ratio of organic: aqueous compounds of the mobile phase. The flow rate was varied by (±) 0.1 ml/min, proportions of methanol in mobile phase varied (80 ± 2) and pH of mobile phase was varied by (±) 0.1. The results are shown in Table No. 3.

4 RESULTS AND DISCUSSION

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment. The linear regression data for the calibration curves showed good linear relationship over the concentration range 20-100

µg/ml for Sitagliptin and 10-50 µg/ml for Simvastatin. Linear regression equation was found to be y = 1.845x + 7.1 (R^2 = 0.998) of SITA and for SIMVA Linear regression equation was found to be y = 9.28x + 22.4 (R^2 = 0.999);shown in fig no 5 and6. The result is expressed in table no 1. The result of specificity was shown in table no 2; the retention times of Sitagliptin and Simvastatin in the sample solution were confirmed by comparing with that of the respective standards. The chromatogram of Tablet sample showed only two peaks at retention time of 2.38 and 7.00 mins for Sitagliptin and Simvastatin respectively, indicating that there is no interference of the excipients in the Tablet formulation.

Repeatability was determined by analyzing 20 µg/ml concentration of Sitagliptin solution and 8 µg/ml concentration of Simvastatin for six times and the % amount found was between 105.3% to 99.73% with % R.S.D. less than 1. Robustness of the developed method result shown in table no3. Results of recovery studies are reported in table 4 which showed that the % amount found was 98.49% and 98.51% with %R.S.D. >2 of SITA and SIMVA. The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation shown in table no 5.The LOD and LOQ for Sitagliptin were found to be 0.61µg and 0.28µg, and The LOD and LOQ for Simvastatin were found to be 1.85 µg and 0.93µg; shown in table no.1

5 CONCLUSION

It is thus concluded that the proposed method is new, simple, cost effective, accurate, safe, free from pollution and precise and can be successfully employed in the routine analysis of these drugs in pharmaceutical dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested noninterference of formulation excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Sitagliptin and Simvastatin. The proposed method shall prove equally effective to analyze Sitagliptin and Simvastatin in the corresponding drug sample and may prove to be of great importance in pharmaceutical analysis.

6 ACKNOWLEDGEMENTS

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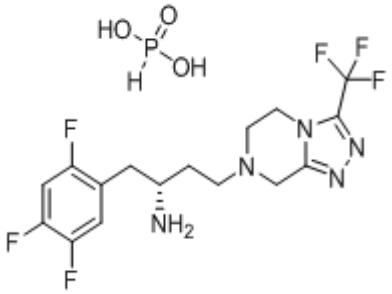

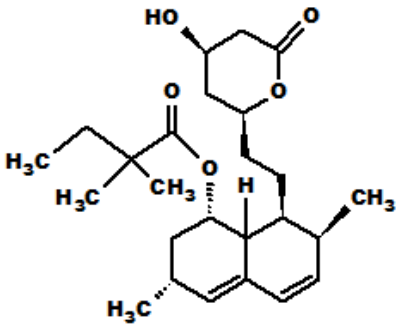

Figure 1: Structure of Sitagliptin Phosphate **Figure 2: Structure of Simvastatin**

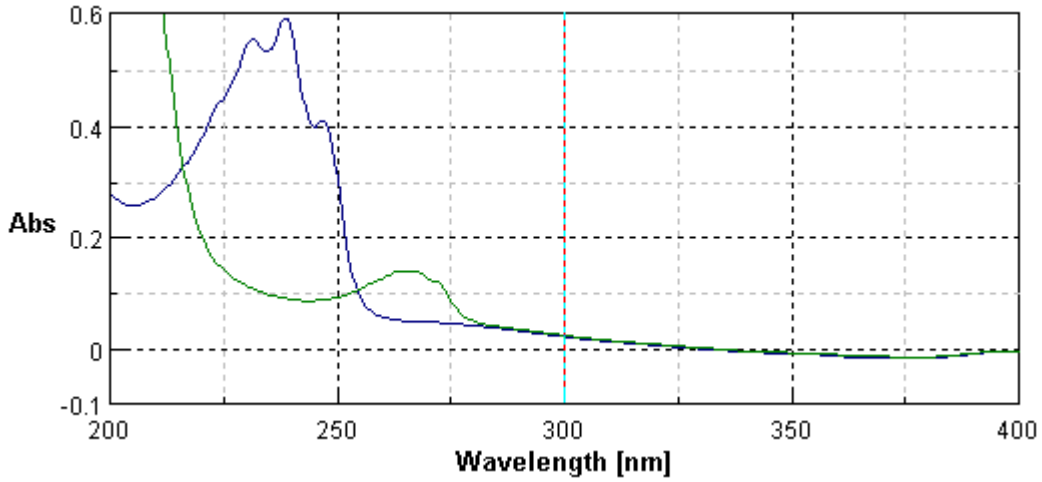

Figure 3: UV Spectrum of Sitagliptin and Simvastatin at isobestic point 254nm

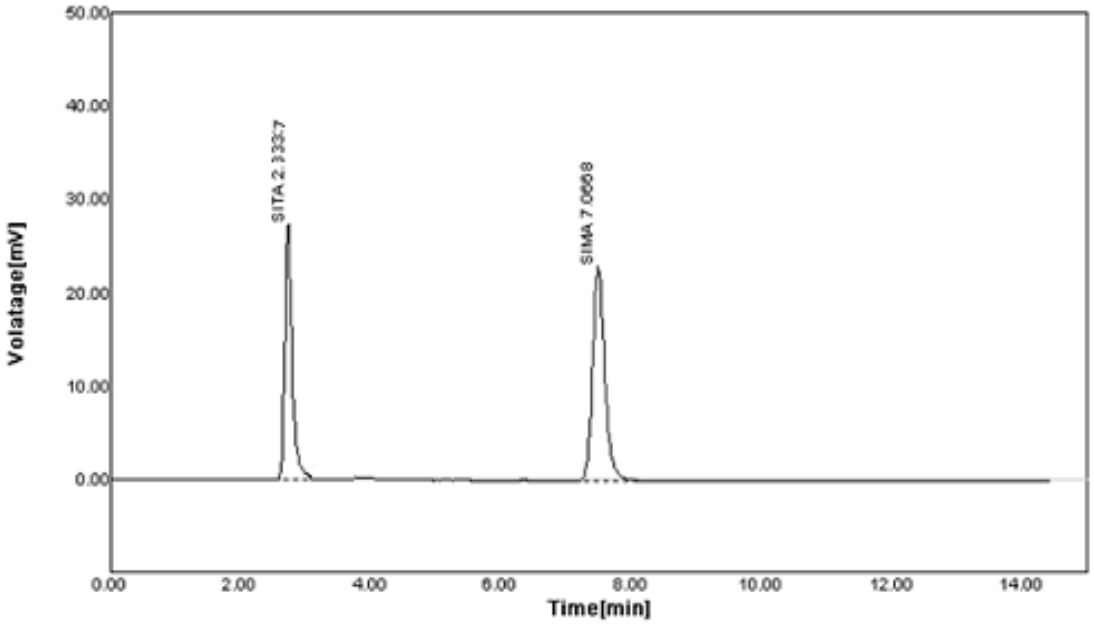

Figure 4: Optimized chromatogram of sitagliptin and simvastatin by RP-HPLC

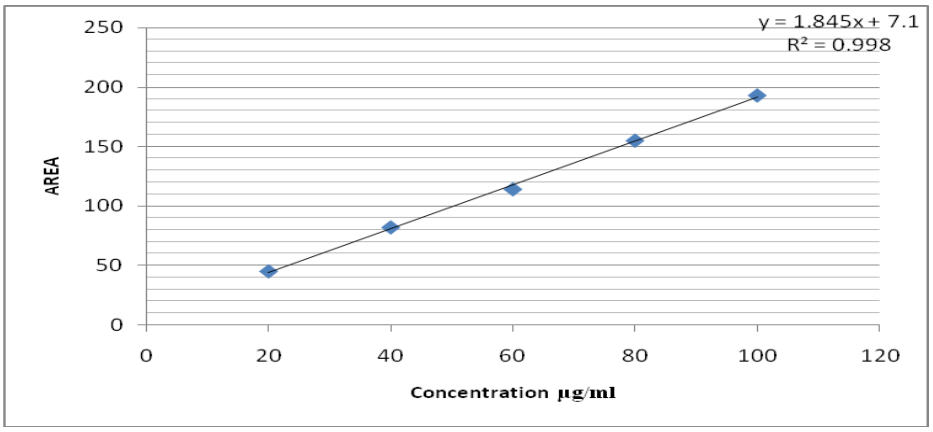

Figure.5: Standard calibration curve of Sitagliptin

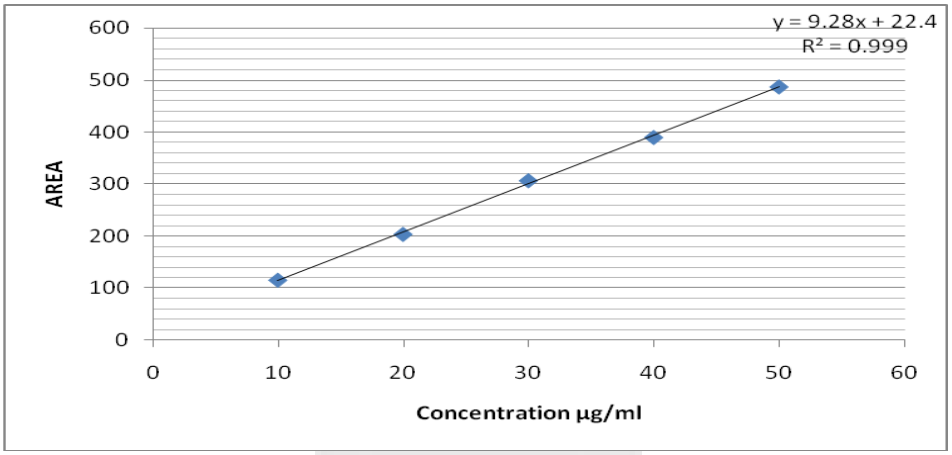

Figure 6: Standard calibration curve of Simvastatin

Table 1: Validation parameter

Parameters	SITA	SIMVA
Slope*	1.845	9.28
Intercept*	7.1	22.4
Correlation coefficient*	0.998	0.999
Linearity range (µg/ml)	20-100	10-50
Accuracy (% recovery± *SD)	98.49±0.007	98.51±0.007
Precision (% CV± *SD)	103.3±0.13	100.04±0.07
Repeatability (% mean± *SD)	105.3±0.13	99.73±0.008
LOD*	0.61	0.28
LOD*	1.85	0.93

*Average of six determinations

Table 2: Result of Specificity

Flow rate	Retention time		Tailing factor		Area		% Content± *SD	
	SITA	SIM	SITA	SIM	SITA	SIM	SITA	SIM
1.0	2.38	7.00	1.87	1.27	72.7450	226.3976	99.89±0.0051	100.46±0.011

*Average of six determination

Table 3: Result of Robustness

P^H	Flow rate	Mobile phase conc.	Level	Retention time		Tailing factor		Area		% Content± *SD	
				SIMA	SITA	SIM	SITA	SIMVA	SITA	SIMVA	SITA
4.1	0.9	79:21	-1	7.94	2.30	1.04	1.82	225.3754	70.776	100.29±0.026	99.29 ±0.04
4.2	1.0	80:20	0	7.00	2.38	1.27	1.87	226.3976	72.745	100.46 ±0.007	99.89 ±0.007
4.3	1.1	81:19	1	7.91	2.28	1.30	1.89	228.8364	73.874	100.60 ±0.005	99.77 ±0.011

*Average of six determinations

Table 4: Result of Accuracy

Ingredients	Tablet amount (µg/ml)	Amount added (µg/ml)	Level of addition	Amount recovered (µg/ml)	Percentage recovery	Average % recovery± *SD
SITA	20(µg/ml) 20(µg/ml) 20(µg/ml)	16(µg/ml) 20(µg/ml) 24(µg/ml)	80% 100% 120%	36(µg/ml) 40 (µg/ml) 44 (µg/ml)	79.89 % 99.35 % 121.4%	100.2%±0.007
SIMVA	8(µg/ml) 8(µg/ml) 8(µg/ml)	6.4(µg/ml) 8(µg/ml) 9.6(µg/ml)	80% 100% 120%	14.4(µg/ml) 16(µg/ml) 17.6(µg/ml)	79.08% 99.45% 120.15%	99.56%±0.011

*Average of six determinations

Table 5: Result of Precision

Drugs	Conc. (µg/ml)	Intra-day		Inter-day	
		*% Mean	*% R.S.D	*% Mean	*% R.S.D
SITA	20	103.3	0.13	99.99	0.014
SIMVA	8	100.04	0.07	100.0	0.13

*Average of six determinations